BIOMARKERS AND METHODS FOR DETERMINING SENSITIVITY TO EPIDERMAL GROWTH FACTOR RECEPTOR MODULATORS IN NON-SMALL CELL LUNG CANCER

SEQUENCE LISTING:

A compact disc labeled "Copy 1" contains the Sequence Listing as 10219 PCT.ST25.txt. The Sequence Listing is 1452 KB in size and was recorded March 24, 2005. The compact disk is 1 of 2 compact disks. A duplicate copy of the compact disc is labeled "Copy 2" and is 2 of 2 compact discs.

The compact disc and duplicate copy are identical and are hereby incorporated by reference into the present application.

FIELD OF THE INVENTION

The present invention relates generally to the field of pharmacogenomics, and more specifically to methods and procedures to determine drug sensitivity in patients to allow the identification of individualized genetic profiles which will aid in treating diseases and disorders.

BACKGROUND OF THE INVENTION:

Cancer is a disease with extensive histoclinical heterogeneity. Although conventional histological and clinical features have been correlated to prognosis, the same apparent prognostic type of tumors varies widely in its responsiveness to therapy and consequent survival of the patient.

New prognostic and predictive markers, which would facilitate an individualization of therapy for each patient, are needed to accurately predict patient response to treatments, such as small molecule or biological molecule drugs, in the clinic. The problem may be solved by the identification of new parameters that could better predict the patient's sensitivity to treatment. The classification of patient samples is a crucial aspect of cancer diagnosis and treatment. The association of a patient's response to a treatment with molecular and genetic markers can open up new opportunities for treatment development in non-responding patients, or distinguish a treatment's indication among other treatment choices because of higher confidence in the efficacy. Further, the pre-selection of patients who are likely to respond well to a medicine, drug, or combination therapy may reduce the number of patients needed in

a clinical study or accelerate the time needed to complete a clinical development program (M. Cockett et al., 2000, *Current Opinion in Biotechnology*, 11:602-609).

The ability to predict drug sensitivity in patients is particularly challenging because drug responses reflect not only properties intrinsic to the target cells, but also a host's metabolic properties. Efforts to use genetic information to predict drug sensitivity have primarily focused on individual genes that have broad effects, such as the multidrug resistance genes, *mdr1* and *mrp1* (P. Sonneveld, 2000, *J. Intern. Med.*, 247:521-534).

The development of microarray technologies for large scale characterization of gene mRNA expression pattern has made it possible to systematically search for molecular markers and to categorize cancers into distinct subgroups not evident by traditional histopathological methods (J. Khan et al., 1998, *Cancer Res.*, 58:5009-5013; A.A. Alizadeh et al., 2000, *Nature*, 403:503-511; M. Bittner et al., 2000, *Nature*, 406:536-540; J. Khan et al., 2001, *Nature Medicine*, 7(6):673-679; and T.R. Golub et al., 1999, *Science*, 286:531-537; U. Alon et al., 1999, *Proc. Natl. Acad. Sci. USA,* 96:6745-6750). Such technologies and molecular tools have made it possible to monitor the expression level of a large number of transcripts within a cell population at any given time (see, e.g., Schena et al., 1995, *Science*, 270:467-470; Lockhart et al., 1996, *Nature Biotechnology*, 14:1675-1680; Blanchard et al., 1996, *Nature Biotechnology*, 14:1649; U.S. Patent No. 5,569,588 to Ashby et al.).

Recent studies demonstrate that gene expression information generated by microarray analysis of human tumors can predict clinical outcome (L.J. van't Veer et al., 2002, *Nature*, 415:530-536; T. Sorlie et al., 2001, *Proc. Natl. Acad. Sci. USA,* 98:10869-10874; M. Shipp et al., 2002, *Nature Medicine*, 8(1):68-74: G.Glinsky et al., 2004, *The Journal of Clin. Invest.*, 113(6):913-923). These findings bring hope that cancer treatment will be vastly improved by better predicting the response of individual tumors to therapy.

Needed are new and alternative methods and procedures to determine drug sensitivity in patients to allow the development of individualized genetic profiles which are necessary to treat diseases and disorders based on patient response at a molecular level.

SUMMARY OF THE INVENTION:

The invention provides methods and procedures for determining patient sensitivity to one or more Epidermal Growth Factor Receptor (EGFR) modulators. The invention also provides methods of determining or predicting whether an individual requiring therapy for a disease state such as cancer will or will not respond to treatment, prior to administration of the treatment, wherein the treatment comprises of one or more EGFR modulators. The one or more EGFR modulators are compounds that can be selected from, for example, one or more EGFR-specific ligands, one or more small molecule EGFR inhibitors, or one or more EGFR binding monoclonal antibodies.

In one aspect, the invention provides a method for identifying a mammal that will respond therapeutically to a method of treating cancer comprising administering of an EGFR modulator, wherein the method comprises: (a) measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1; (b) exposing a biological sample from the mammal to the EGFR modulator; (c) following the exposing in step (b), measuring in said biological sample the level of the at least one biomarker, wherein a difference in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates that the mammal will respond therapeutically to the said method of treating cancer.

A difference in the level of the biomarker that is sufficient to indicate whether the mammal will or will not respond therapeutically to the method of treating cancer can be readily determined by one of skill in the art using known techniques. The increase or decrease in the level of the biomarker can be correlated to determine whether the difference is sufficient to identify a mammal that will respond therapeutically. The difference in the level of the biomarker that is sufficient can, in one aspect, be predetermined prior to determining whether the mammal will respond therapeutically to the treatment. In one aspect, the difference in the level of the biomarker is a difference in the mRNA level (measured, for example, by RT-PCT or a microarray), such as at least a two-fold difference, at least a three-fold difference, or at least a four-fold difference in the level of expression. In another aspect, the difference in the level of the biomarker is determined by IHC. In another aspect, the

difference in the level of the biomarker refers to a p-value of <0.05 in Anova analysis. In yet another aspect, the difference is determined in an ELISA assay.

As used herein, respond therapeutically refers to the alleviation or abrogation of the cancer. This means that the life expectancy of an individual affected with the cancer will be increased or that one or more of the symptoms of the cancer will be reduced or ameliorated. The term encompasses a reduction in cancerous cell growth or tumor volume. Whether a mammal responds therapeutically can be measured by many methods well known in the art, such as PET imaging.

The mammal can be, for example, a human, rat, mouse, dog, rabbit, pig sheep, cow, horse, cat, primate, or monkey.

The method of the invention can be, for example, an *in vitro* method wherein the step of measuring in the mammal the level of at least one biomarker comprises taking a biological sample from the mammal and then measuring the level of the at least one biomarker in the biological sample. The biological sample can comprise, for example, at least one of serum, whole fresh blood, peripheral blood mononuclear cells, frozen whole blood, fresh plasma, frozen plasma, urine, saliva, skin, hair follicle, bone marrow, or tumor tissue.

The level of the at least one biomarker can be, for example, the level of protein and/or mRNA transcript of the at least one biomarker.

In another aspect, the invention provides a method for identifying a mammal that will respond therapeutically to a method of treating cancer comprising administering an EGFR modulator, wherein the method comprises: (a) exposing a biological sample from the mammal to the EGFR modulator; (b) following the exposing of step (a), measuring in said biological sample the level of at least one biomarker selected from the biomarkers of Table 1, wherein a difference in the level of the at least one biomarker measured in step (b), compared to the level of the at least one biomarker in a mammal that has not been exposed to said EGFR modulator, indicates that the mammal will respond therapeutically to said method of treating cancer.

In yet another aspect, the invention provides a method for testing or predicting whether a mammal will respond therapeutically to a method of treating cancer comprising administering an EGFR modulator, wherein the method comprises: (a)

measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1; (b) exposing the mammal to the EGFR modulator; (c) following the exposing of step (b), measuring in the mammal the level of the at least one biomarker, wherein a difference in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates that the mammal will respond therapeutically to said method of treating cancer.

In another aspect, the invention provides a method for determining whether a compound inhibits EGFR activity in a mammal, comprising: (a) exposing the mammal to the compound; and (b) following the exposing of step (a), measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1, wherein a difference in the level of said biomarker measured in step (b), compared to the level of the biomarker in a mammal that has not been exposed to said compound, indicates that the compound inhibits EGFR activity in the mammal.

In yet another aspect, the invention provides a method for determining whether a mammal has been exposed to a compound that inhibits EGFR activity, comprising (a) exposing the mammal to the compound; and (b) following the exposing of step (a), measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1, wherein a difference in the level of said biomarker measured in step (b), compared to the level of the biomarker in a mammal that has not been exposed to said compound, indicates that the mammal has been exposed to a compound that inhibits EGFR activity.

In another aspect, the invention provides a method for determining whether a mammal is responding to a compound that inhibits EGFR activity, comprising (a) exposing the mammal to the compound; and (b) following the exposing of step (a), measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1, wherein a difference in the level of the at least one biomarker measured in step (b), compared to the level of the at least one biomarker in a mammal that has not been exposed to said compound, indicates that the mammal is responding to the compound that inhibits EGFR activity.

As used herein, "responding" encompasses responding by way of a biological and cellular response, as well as a clinical response (such as improved symptoms, a therapeutic effect, or an adverse event), in a mammal.

The invention also provides an isolated biomarker selected from the biomarkers of Table 1. The biomarkers of the invention comprise sequences selected from the nucleotide and amino acid sequences provided in Table 1 and the Sequence Listing, as well as fragments and variants thereof.

The invention also provides a biomarker set comprising two or more biomarkers selected from the biomarkers of Table 1.

The invention also provides kits for determining or predicting whether a patient would be susceptible or resistant to a treatment that comprises one or more EGFR modulators. The patient may have a cancer or tumor such as, for example, a non-small cell lung cancer (NSCLC) or tumor.

In one aspect, the kit comprises a suitable container that comprises one or more specialized microarrays of the invention, one or more EGFR modulators for use in testing cells from patient tissue specimens or patient samples, and instructions for use. The kit may further comprise reagents or materials for monitoring the expression of a biomarker set at the level of mRNA or protein.

In another aspect, the invention provides a kit comprising two or more biomarkers selected from the biomarkers of Table 1.

In yet another aspect, the invention provides a kit comprising at least one of an antibody and a nucleic acid for detecting the presence of at least one of the biomarkers selected from the biomarkers of Table 1. In one aspect, the kit further comprises instructions for determining whether or not a mammal will respond therapeutically to a method of treating cancer comprising administering a compound that inhibits EGFR activity. In another aspect, the instructions comprise the steps of (a) measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1, (b) exposing the mammal to the compound, (c) following the exposing of step (b), measuring in the mammal the level of the at least one biomarker, wherein a difference in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates that the mammal will respond therapeutically to said method of treating cancer.

The invention also provides screening assays for determining if a patient will be susceptible or resistant to treatment with one or more EGFR modulators.

The invention also provides a method of monitoring the treatment of a patient having a disease, wherein said disease is treated by a method comprising administering one or more EGFR modulators.

The invention also provides individualized genetic profiles which are necessary to treat diseases and disorders based on patient response at a molecular level.

The invention also provides specialized microarrays, e.g., oligonucleotide microarrays or cDNA microarrays, comprising one or more biomarkers having expression profiles that correlate with either sensitivity or resistance to one or more EGFR modulators.

The invention also provides antibodies, including polyclonal or monoclonal, directed against one or more biomarkers of the invention.

The invention will be better understood upon a reading of the detailed description of the invention when considered in connection with the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES:

FIG. 1 illustrates the scheme used for identifying the Table 1 biomarkers.

FIG. 2 illustrates the scheme used for identifying the Table 2 biomarkers.

FIG. 3 shows the mRNA levels of EGFR determined by expression profiling of fourteen NSCLC cell lines.

FIG. 4 illustrates the variance analysis of expression profiles.

FIG. 5 illustrates the variance metric distribution of probe sets for the adenocarcinoma tumors.

FIG. 6 illustrates the variance metric distribution of probe sets for the cell lines.

FIG. 7 illustrates the scoring of staining of a Calgranulin B IHC Assay.

DETAILED DESCRIPTION OF THE INVENTION:

Identification of biomarkers that provide rapid and accessible readouts of efficacy, drug exposure, or clinical response is increasingly important in the clinical development of drug candidates. Embodiments of the invention include measuring changes in the levels of secreted proteins, or plasma biomarkers, which represent one category of biomarker. In one aspect, plasma samples, which represent a readily accessible source of material, serves a surrogate tissue for biomarker analysis.

The invention provides biomarkers that respond to the modulation of a specific signal transduction pathway and also correlate with EGFR modulator sensitivity or resistance. These biomarkers can be employed for predicting response to one or more EGFR modulators. In one aspect, the biomarkers of the invention are those provided in Table 1 and the Sequence Listing, including both polynucleotide and polypeptide sequences.

TABLE 1 - Biomarkers

Unigene title and SEQ ID NO:	Affymetrix Description	Affymetrix Probe Set
S100A14: S100 calcium binding protein A14 (LOC57402) SEQ ID NOS: 1 (DNA) and 148 (amino acid)	gb:NM_020672.1 /DEF=Homo sapiens S100-type calcium binding protein A14 (LOC57402), mRNA. /FEA=mRNA /GEN=LOC57402 /PROD=S100-type calcium binding protein A14 /DB_XREF=gi:10190711 /UG=Hs.288998 S100-type calcium binding protein A14 /FL=gb:NM_020672.1 gb:BC005019.1 gb:AY007220.1	218677_at
JTB: jumping translocation breakpoint (LOC10899) SEQ ID NOS: 2 (DNA) and 149 (amino acid)	gb:AF151056.1 /DEF=Homo sapiens HSPC222 mRNA, complete cds. /FEA=mRNA /PROD=HSPC222 /DB_XREF=gi:7106833 /UG=Hs.323093 Homo sapiens, jumping translocation breakpoint, clone MGC:10274, mRNA, complete cds /FL=gb:AF151056.1	210434_x_at
CDH1: cadherin 1, type 1 preproprotein (LOC999) SEQ ID NOS: 3 (DNA) and 150 (amino acid)	gb:NM_004360.1 /DEF=Homo sapiens cadherin 1, type 1, E-cadherin (epithelial) (CDH1), mRNA. /FEA=mRNA /GEN=CDH1 /PROD=cadherin 1, type 1, E-cadherin (epithelial) /DB_XREF=gi:4757959 /UG=Hs.194657 cadherin 1, type 1, E-cadherin (epithelial) /FL=gb:L08599.1 gb:NM_004360.1	201131_s_at

CYR61: cysteine-rich, angiogenic inducer, 61 (LOC3491) SEQ ID NOS: 4 (DNA) and 151 (amino acid)	gb:NM_001554.1 /DEF=Homo sapiens cysteine-rich, angiogenic inducer, 61 (CYR61), mRNA. /FEA=mRNA /GEN=CYR61 /PROD=cysteine-rich, angiogenic inducer, 61 /DB_XREF=gi:4504612 /UG=Hs.8867 cysteine-rich, angiogenic inducer, 61 /FL=gb:BC001271.1 gb:U62015.1 gb:AF003594.1 gb:AF031385.1 gb:NM_001554.1	201289_at
TGFBI: transforming growth factor, beta-induced, 68kDa (LOC7045) SEQ ID NOS: 5 (DNA) and 152 (amino acid)	gb:NM_000358.1 /DEF=Homo sapiens transforming growth factor, beta-induced, 68kD (TGFBI), mRNA. /FEA=mRNA /GEN=TGFBI /PROD=transforming growth factor, beta-induced, 68kD /DB_XREF=gi:4507466 /UG=Hs.118787 transforming growth factor, beta-induced, 68kD /FL=gb:BC000097.1 gb:BC004972.1 gb:M77349.1 gb:NM_000358.1	201506_at
PSPHL: phosphoserine phosphatase-like (LOC8781) SEQ ID NOS: 6 (DNA) and 153 (amino acid)	Consensus includes gb:BF968134 /FEA=EST /DB_XREF=gi:12335349 /DB_XREF=est:602269121F1 /CLONE=IMAGE:4357349 /UG=Hs.250723 FK506 binding protein 12-rapamycin associated protein 1	212509_s_at
DKK1: dickkopf homolog 1 (LOC22943) SEQ ID NOS: 7 (DNA) and 154 (amino acid)	gb:NM_012242.1 /DEF=Homo sapiens dickkopf (Xenopus laevis) homolog 1 (DKK1), mRNA. /FEA=mRNA /GEN=DKK1 /PROD=dickkopf (Xenopus laevis) homolog 1 /DB_XREF=gi:7110718 /UG=Hs.40499 dickkopf (Xenopus laevis) homolog 1 /FL=gb:AF127563.1 gb:AF177394.1 gb:NM_012242.1	204602_at
FHL1: four and a half LIM domains 1 (LOC2273) SEQ ID NOS: 8 (DNA) and 155 (amino acid)	gb:NM_001449.1 /DEF=Homo sapiens four and a half LIM domains 1 (FHL1), mRNA. /FEA=mRNA /GEN=FHL1 /PROD=four and a half LIM domains 1 /DB_XREF=gi:4503720 /UG=Hs.239069 four and a half LIM domains 1 /FL=gb:U29538.1 gb:U60115.1 gb:NM_001449.1	201540_at

SSR4: signal sequence receptor, delta (LOC6748) SEQ ID NOS: 9 (DNA) and 156 (amino acid)	gb:NM_006280.1 /DEF=Homo sapiens signal sequence receptor, delta (translocon-associated protein delta) (SSR4), mRNA. /FEA=mRNA /GEN=SSR4 /PROD=signal sequence receptor, delta /DB_XREF=gi:5454089 /UG=Hs.102135 signal sequence receptor, delta (translocon-associated protein delta) /FL=gb:BC003371.1 gb:NM_006280.1	201004_at
S100A9: S100 calcium-binding protein A9 (LOC6280) SEQ ID NOS: 10 (DNA) and 157 (amino acid)	gb:NM_002965.2 /DEF=Homo sapiens S100 calcium-binding protein A9 (calgranulin B) (S100A9), mRNA. /FEA=mRNA /GEN=S100A9 /PROD=S100 calcium-binding protein A9 /DB_XREF=gi:9845520 /UG=Hs.112405 S100 calcium-binding protein A9 (calgranulin B) /FL=gb:M26311.1 gb:NM_002965.2	203535_at
SFN: stratifin (LOC2810) SEQ ID NOS: 11 (DNA) and 158 (amino acid)	Cluster Incl. X57348:H.sapiens mRNA (clone 9112) /cds=(165,911) /gb=X57348 /gi=23939 /ug=Hs.184510 /len=1407	33322_i_at
F2RL1: coagulation factor II (thrombin) receptor-like 1 precursor (LOC2150) SEQ ID NOS: 12 (DNA) and 159 (amino acid)	Consensus includes gb:BE965369 /FEA=EST /DB_XREF=gi:11769659 /DB_XREF=est:601659282R1 /CLONE=IMAGE:3895653 /UG=Hs.168102 Human proteinase activated receptor-2 mRNA, 3UTR	213506_at
SPUVE: protease, serine, 23 precursor (LOC11098) SEQ ID NOS: 13 (DNA) and 160 (amino acid)	gb:NM_007173.1 /DEF=Homo sapiens protease, serine, 23 (SPUVE), mRNA. /FEA=mRNA /GEN=SPUVE /PROD=protease, serine, 23 /DB_XREF=gi:6005881 /UG=Hs.325820 protease, serine, 23 /FL=gb:AL136914.1 gb:BC001278.1 gb:AF015287.1 gb:NM_007173.1 gb:AF193611.1	202458_at
AMIGO2: amphoterin induced gene 2 (LOC347902) SEQ ID NOS: 14 (DNA) and 161 (amino acid)	Consensus includes gb:AC004010 /DEF=Human BAC clone GS1-99H8 /FEA=CDS /DB_XREF=gi:2781385 /UG=Hs.121520 Human BAC clone GS1-99H8	222108_at

KRT7: keratin 7 (LOC3855) SEQ ID NOS: 15 (DNA) and 162 (amino acid)	gb:BC002700.1 /DEF=Homo sapiens, Similar to keratin 7, clone MGC:3625, mRNA, complete cds. /FEA=mRNA /PROD=Similar to keratin 7 /DB_XREF=gi:12803726 /UG=Hs.23881 keratin 7 /FL=gb:BC002700.1 gb:NM_005556.1	209016_s_at
RPL13: ribosomal protein L13 (LOC6137) SEQ ID NOS: 16 (DNA) and 163 (amino acid)	Consensus includes gb:AW574664 /FEA=EST /DB_XREF=gi:7246203 /DB_XREF=est:UI-HF-BL0-abw-d-10-0-UI.s1 /CLONE=IMAGE:3057859 /UG=Hs.180842 ribosomal protein L13	212191_x_at
AF1Q: AF1Q protein (LOC10962) SEQ ID NOS: 17 (DNA) and 164 (amino acid)	gb:BC006471.1 /DEF=Homo sapiens, ALL1-fused gene from chromosome 1q, clone MGC:4013, mRNA, complete cds. /FEA=mRNA /PROD=ALL1-fused gene from chromosome 1q /DB_XREF=gi:13623686 /FL=gb:BC006471.1	211071_s_at
COL6A2: alpha 2 type VI collagen isoform 2C2 precursor (LOC1292) SEQ ID NOS: 18 (DNA) and 165 (amino acid)	gb:AY029208.1 /DEF=Homo sapiens type VI collagen alpha 2 chain precursor (COL6A2) mRNA, complete cds, alternatively spliced. /FEA=mRNA /GEN=COL6A2 /PROD=type VI collagen alpha 2 chain precursor /DB_XREF=gi:13603393 /UG=Hs.159263 collagen, type VI, alpha 2 /FL=gb:AY029208.1	209156_s_at
COL6A1: collagen, type VI, alpha 1 precursor (LOC1291) SEQ ID NOS: 19 (DNA) and 166 (amino acid)	Consensus includes gb:AA292373 /FEA=EST /DB_XREF=gi:1940353 /DB_XREF=est:zt51a09.s1 /CLONE=IMAGE:725848 /UG=Hs.108885 collagen, type VI, alpha 1	213428_s_at
SLC38A2: solute carrier family 38, member 2 (LOC54407) SEQ ID NOS: 20 (DNA) and 167 (amino acid)	gb:NM_018573.1 /DEF=Homo sapiens hypothetical protein PRO1068 (PRO1068), mRNA. /FEA=mRNA /GEN=PRO1068 /PROD=hypothetical protein PRO1068 /DB_XREF=gi:8924006 /UG=Hs.321158 hypothetical protein PRO1068 /FL=gb:AF116620.1 gb:NM_018573.1	218041_x_at

PAPSS2: 3'-phosphoadenosine 5'-phosphosulfate synthase 2 (LOC9060) SEQ ID NOS: 21 (DNA) and 168 (amino acid)	gb:AF074331.1 /DEF=Homo sapiens PAPS synthetase-2 (PAPSS2) mRNA, complete cds. /FEA=mRNA /GEN=PAPSS2 /PROD=PAPS synthetase-2 /DB_XREF=gi:5052074 /UG=Hs.274230 3-phosphoadenosine 5-phosphosulfate synthase 2 /FL=gb:AF150754.2 gb:AF313907.1 gb:AF091242.1 gb:NM_004670.1 gb:AF074331.1 gb:AF173365.1	203060_s_at
JAG1: jagged 1 precursor (LOC182) SEQ ID NOS: 22 (DNA) and 169 (amino acid)	gb:U73936.1 /DEF=Homo sapiens Jagged 1 (HJ1) mRNA, complete cds. /FEA=mRNA /GEN=HJ1 /PROD=Jagged 1 /DB_XREF=gi:1695273 /UG=Hs.91143 jagged 1 (Alagille syndrome) /FL=gb:U61276.1 gb:U73936.1 gb:AF003837.1 gb:AF028593.1 gb:NM_000214.1	209099_x_at
RPS27L: ribosomal protein S27-like protein (LOC51065) SEQ ID NOS: 23 (DNA) and 170 (amino acid)	gb:NM_015920.1 /DEF=Homo sapiens 40S ribosomal protein S27 isoform (LOC51065), mRNA. /FEA=mRNA /GEN=LOC51065 /PROD=40S ribosomal protein S27 isoform /DB_XREF=gi:7705705 /UG=Hs.108957 40S ribosomal protein S27 isoform /FL=gb:BC003667.1 gb:AF070668.1 gb:NM_015920.1	218007_s_at
PAM: peptidylglycine alpha-amidating monooxygenase isoform a, preproprotein (LOC5066) SEQ ID NOS: 24 (DNA) and 171 (amino acid)	gb:NM_000919.1 /DEF=Homo sapiens peptidylglycine alpha-amidating monooxygenase (PAM), mRNA. /FEA=mRNA /GEN=PAM /PROD=peptidylglycine alpha-amidating monooxygenase /DB_XREF=gi:4505602 /UG=Hs.83920 peptidylglycine alpha-amidating monooxygenase /FL=gb:M37721.1 gb:NM_000919.1	202336_s_at
STAT1: signal transducer and activator of transcription 1 isoform alpha (LOC6772) SEQ ID NOS: 25 (DNA) and 172 (amino acid)	gb:NM_007315.1 /DEF=Homo sapiens signal transducer and activator of transcription 1, 91kD (STAT1), mRNA. /FEA=mRNA /GEN=STAT1 /PROD=signal transducer and activator of transcription1, 91kD /DB_XREF=gi:6274551 /UG=Hs.21486 signal transducer and activator of transcription 1, 91kD /FL=gb:M97935.1 gb:NM_007315.1	200887_s_at

CTSB: cathepsin B preproprotein (LOC1508) SEQ ID NOS: 26 (DNA) and 173 (amino acid)	gb:NM_001908.1 /DEF=Homo sapiens cathepsin B (CTSB), mRNA. /FEA=mRNA /GEN=CTSB /PROD=cathepsin B /DB_XREF=gi:4503138 /UG=Hs.297939 cathepsin B /FL=gb:M14221.1 gb:L16510.1 gb:NM_001908.1	200839_s_at
POLR2L: DNA directed RNA polymerase II polypeptide L (LOC5441) SEQ ID NOS: 27 (DNA) and 174 (amino acid)	gb:BC005903.1 /DEF=Homo sapiens, polymerase (RNA) II (DNA directed) polypeptide L (7.6kD), clone MGC:14494, mRNA, complete cds. /FEA=mRNA /PROD=polymerase (RNA) II (DNA directed) polypeptide L(7.6kD) /DB_XREF=gi:13543491 /FL=gb:BC005903.1	211730_s_at
ETV1: ets variant gene 1 (LOC2115) SEQ ID NOS: 28 (DNA) and 175 (amino acid)	Consensus includes gb:BE881590 /FEA=EST /DB_XREF=gi:10330366 /DB_XREF=est:601490008F1 /CLONE=IMAGE:3892465 /UG=Hs.10684 Homo sapiens clone 24421 mRNA sequence	221911_at
KRT18: keratin 18 (LOC3875) SEQ ID NOS: 29 (DNA) and 176 (amino acid)	gb:NM_000224.1 /DEF=Homo sapiens keratin 18 (KRT18), mRNA. /FEA=mRNA /GEN=KRT18 /PROD=keratin 18 /DB_XREF=gi:4557887 /UG=Hs.65114 keratin 18 /FL=gb:BC000698.1 gb:BC000180.2 gb:BC004253.1 gb:M26326.1 gb:NM_000224.1	201596_x_at
RPL29: ribosomal protein L29 (LOC6159) SEQ ID NOS: 30 (DNA) and 177 (amino acid)	Consensus includes gb:BF683426 /FEA=EST /DB_XREF=gi:11968834 /DB_XREF=est:602139603F1 /CLONE=IMAGE:4300777 /UG=Hs.183698 ribosomal protein L29	213969_x_at
PYGB: brain glycogen phosphorylase (LOC5834) SEQ ID NOS: 31 (DNA) and 178 (amino acid)	gb:NM_002862.1 /DEF=Homo sapiens phosphorylase, glycogen; brain (PYGB), nuclear gene encoding mitochondrial protein, mRNA. /FEA=mRNA /GEN=PYGB /PROD=phosphorylase, glycogen; brain /DB_XREF=gi:4506350 /UG=Hs.75658 phosphorylase, glycogen; brain /FL=gb:U47025.1 gb:NM_002862.1	201481_s_at

ALCAM: activated leukocyte cell adhesion molecule (LOC214) SEQ ID NOS: 32 (DNA) and 179 (amino acid)	Consensus includes gb:AA156721 /FEA=EST /DB_XREF=gi:1728335 /DB_XREF=est:zl18b04.s1 /CLONE=IMAGE:502255 /UG=Hs.10247 activated leucocyte cell adhesion molecule /FL=gb:NM_001627.1 gb:L38608.1	201952_at
CTGF: connective tissue growth factor (LOC1490) SEQ ID NOS: 33 (DNA) and 180 (amino acid)	gb:M92934.1 /DEF=Human connective tissue growth factor, complete cds. /FEA=mRNA /PROD=connective tissue growth factor /DB_XREF=gi:180923 /UG=Hs.75511 connective tissue growth factor /FL=gb:M92934.1 gb:NM_001901.1	209101_at
UCHL1: ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase) (LOC7345) SEQ ID NOS: 34 (DNA) and 181 (amino acid)	gb:NM_004181.1 /DEF=Homo sapiens ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase) (UCHL1), mRNA. /FEA=mRNA /GEN=UCHL1 /PROD=ubiquitin carboxyl-terminal esterase L1(ubiquitin thiolesterase) /DB_XREF=gi:4759283 /UG=Hs.76118 ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase) /FL=gb:BC000332.1 gb:BC005117.1 gb:NM_004181.1	201387_s_at
C14orf78: chromosome 14 open reading frame 78 (LOC113146) SEQ ID NOS: 35 (DNA) and 182 (amino acid)	Consensus includes gb:AI935123 /FEA=EST /DB_XREF=gi:5673993 /DB_XREF=est:wp13h09.x1 /CLONE=IMAGE:2464769 /UG=Hs.57548 ESTs	212992_at
PBEF: pre-B-cell colony-enhancing factor isoform a (LOC10135) SEQ ID NOS: 36 (DNA) and 183 (amino acid)	Consensus includes gb:BF575514 /FEA=EST /DB_XREF=gi:11649318 /DB_XREF=est:602133090F1 /CLONE=IMAGE:4288079 /UG=Hs.239138 pre-B-cell colony-enhancing factor /FL=gb:U02020.1 gb:NM_005746.1	217738_at

GNG11: guanine nucleotide binding protein (G protein), gamma 11 (LOC2791) SEQ ID NOS: 37 (DNA) and 184 (amino acid)	gb:NM_004126.1 /DEF=Homo sapiens guanine nucleotide binding protein 11 (GNG11), mRNA. /FEA=mRNA /GEN=GNG11 /PROD=guanine nucleotide binding protein 11 /DB_XREF=gi:4758447 /UG=Hs.83381 guanine nucleotide binding protein 11 /FL=gb:NM_004126.1 gb:U31384.1	204115_at
SERPINE2: plasminogen activator inhibitor type 1, member 2 (LOC5270) SEQ ID NOS: 38 (DNA) and 185 (amino acid)	Consensus includes gb:AL541302 /FEA=EST /DB_XREF=gi:12872241 /DB_XREF=est:AL541302 /CLONE=CS0DE006YI10 (5 prime) /UG=Hs.21858 trinucleotide repeat containing 3	212190_at
PTTG1IP: pituitary tumor-transforming gene 1 protein-interacting protein precursor (LOC754) SEQ ID NOS: 39 (DNA) and 186 (amino acid)	gb:NM_004339.2 /DEF=Homo sapiens pituitary tumor-transforming 1 interacting protein (PTTG1IP), mRNA. /FEA=mRNA /GEN=PTTG1IP /PROD=pituitary tumor-transforming protein1-interacting protein precursor /DB_XREF=gi:11038670 /UG=Hs.111126 pituitary tumor-transforming 1 interacting protein /FL=gb:NM_004339.2 gb:BC000415.1 gb:AF149785.1	200677_at
KRT19: keratin 19 (LOC3880) SEQ ID NOS: 40 (DNA) and 187 (amino acid)	gb:NM_002276.1 /DEF=Homo sapiens keratin 19 (KRT19), mRNA. /FEA=mRNA /GEN=KRT19 /PROD=keratin 19 /DB_XREF=gi:4504916 /UG=Hs.182265 keratin 19 /FL=gb:BC002539.1 gb:NM_002276.1	201650_at
SFN: stratifin (LOC2810) SEQ ID NOS: 41 (DNA) and 188 (amino acid)	Cluster Incl. X57348:H.sapiens mRNA (clone 9112) /cds=(165,911) /gb=X57348 /gi=23939 /ug=Hs.184510 /len=1407	33323_r_at
ICAM1: intercellular adhesion molecule 1 (LOC3383) SEQ ID NOS: 42 (DNA) and 189 (amino acid)	Consensus includes gb:AI608725 /FEA=EST /DB_XREF=gi:4617892 /DB_XREF=est:tw90b01.x1 /CLONE=IMAGE:2266921 /UG=Hs.168383 intercellular adhesion molecule 1 (CD54), human rhinovirus receptor /FL=gb:M24283.1 gb:J03132.1 gb:NM_000201.1	202637_s_at

SLC6A8: solute carrier family 6 (neurotransmitter transporter, creatine), member 8 (LOC6535) SEQ ID NOS: 43 (DNA) and 190 (amino acid)	gb:NM_005629.1 /DEF=Homo sapiens solute carrier family 6 (neurotransmitter transporter, creatine), member 8 (SLC6A8), mRNA. /FEA=mRNA /GEN=SLC6A8 /PROD=solute carrier family 6 (neurotransmittertransporter, creatine), member 8 /DB_XREF=gi:5032096 /UG=Hs.187958 solute carrier family 6 (neurotransmitter transporter, creatine), member 8 /FL=gb:L31409.1 gb:NM_005629.1	202219_at
IL8: interleukin 8 (LOC3576) SEQ ID NOS: 44 (DNA) and 191 (amino acid)	gb:AF043337.1 /DEF=Homo sapiens interleukin 8 C-terminal variant (IL8) mRNA, complete cds. /FEA=mRNA /GEN=IL8 /PROD=interleukin 8 C-terminal variant /DB_XREF=gi:12641914 /UG=Hs.624 interleukin 8 /FL=gb:AF043337.1	211506_s_at
CSPG2: chondroitin sulfate proteoglycan 2 (versican) (LOC1462) SEQ ID NOS: 45 (DNA) and 192 (amino acid)	gb:NM_004385.1 /DEF=Homo sapiens chondroitin sulfate proteoglycan 2 (versican) (CSPG2), mRNA. /FEA=mRNA /GEN=CSPG2 /PROD=chondroitin sulfate proteoglycan 2 (versican) /DB_XREF=gi:4758081 /UG=Hs.81800 chondroitin sulfate proteoglycan 2 (versican) /FL=gb:NM_004385.1	204620_s_at
CTSC: cathepsin C isoform a preproprotein (LOC1075) SEQ ID NOS: 46 (DNA) and 193 (amino acid)	gb:NM_001814.1 /DEF=Homo sapiens cathepsin C (CTSC), mRNA. /FEA=mRNA /GEN=CTSC /PROD=cathepsin C /DB_XREF=gi:4503140 /UG=Hs.10029 cathepsin C /FL=gb:NM_001814.1	201487_at
JTB: jumping translocation breakpoint (LOC10899) SEQ ID NOS: 47 (DNA) and 194 (amino acid)	gb:BC004239.1 /DEF=Homo sapiens, jumping translocation breakpoint, clone MGC:10274, mRNA, complete cds. /FEA=mRNA /PROD=jumping translocation breakpoint /DB_XREF=gi:13278986 /UG=Hs.323093 Homo sapiens, jumping translocation breakpoint, clone MGC:10274, mRNA, complete cds /FL=gb:BC004239.1	210927_x_at
KRT8: keratin 8 (LOC3856) SEQ ID NOS: 48 (DNA) and 195 (amino acid)	gb:U76549.1 /DEF=Human cytokeratin 8 mRNA, complete cds. /FEA=mRNA /PROD=cytokeratin 8 /DB_XREF=gi:1673574 /UG=Hs.242463 keratin 8 /FL=gb:BC000654.1 gb:U76549.1 gb:M34225.1 gb:M26324.1 gb:NM_002273.1	209008_x_at

UGDH: UDP-glucose dehydrogenase (LOC7358) SEQ ID NOS: 49 (DNA) and 196 (amino acid)	gb:NM_003359.1 /DEF=Homo sapiens UDP-glucose dehydrogenase (UGDH), mRNA. /FEA=mRNA /GEN=UGDH /PROD=UDP-glucose dehydrogenase /DB_XREF=gi:4507812 /UG=Hs.28309 UDP-glucose dehydrogenase /FL=gb:AF061016.1 gb:NM_003359.1	203343_at
TXNIP: thioredoxin interacting protein (LOC10628) SEQ ID NOS: 50 (DNA) and 197 (amino acid)	Consensus includes gb:AA812232 /FEA=EST /DB_XREF=gi:2881843 /DB_XREF=est:ob84h09.s1 /CLONE=IMAGE:1338113 /UG=Hs.179526 upregulated by 1,25-dihydroxyvitamin D-3 /FL=gb:NM_006472.1 gb:S73591.1	201008_s_at
CTSB: cathepsin B preproprotein (LOC1508) SEQ ID NOS: 51 (DNA) and 198 (amino acid)	gb:NM_001908.1 /DEF=Homo sapiens cathepsin B (CTSB), mRNA. /FEA=mRNA /GEN=CTSB /PROD=cathepsin B /DB_XREF=gi:4503138 /UG=Hs.297939 cathepsin B /FL=gb:M14221.1 gb:L16510.1 gb:NM_001908.1	200838_at
CSPG2: chondroitin sulfate proteoglycan 2 (versican) (LOC1462) SEQ ID NOS: 52 (DNA) and 199 (amino acid)	Consensus includes gb:BF218922 /FEA=EST /DB_XREF=gi:11112418 /DB_XREF=est:601885091F1 /CLONE=IMAGE:4103447 /UG=Hs.81800 chondroitin sulfate proteoglycan 2 (versican)	221731_x_at
ANXA10: annexin A10 (LOC11199) SEQ ID NOS: 53 (DNA) and 200 (amino acid)	gb:AF196478.1 /DEF=Homo sapiens annexin 14 (ANX14) mRNA, complete cds. /FEA=mRNA /GEN=ANX14 /PROD=annexin 14 /DB_XREF=gi:6274496 /UG=Hs.188401 annexin A10 /FL=gb:AF196478.1 gb:NM_007193.2	210143_at
SAT: spermidine/spermine N1-acetyltransferase (LOC6303) SEQ ID NOS: 54 (DNA) and 201 (amino acid)	gb:M55580.1 /DEF=Human spermidinespermine N1-acetyltransferase mRNA, complete cds. /FEA=mRNA /GEN=spermidinespermine N1-acetyltransferase /PROD=spermidinespermine N1-acetyltransferase /DB_XREF=gi:338335 /UG=Hs.28491 spermidinespermine N1-acetyltransferase /FL=gb:M55580.1	210592_s_at

COL6A3: alpha 3 type VI collagen isoform 1 precursor (LOC1293) SEQ ID NOS: 55 (DNA) and 202 (amino acid)	gb:NM_004369.1 /DEF=Homo sapiens collagen, type VI, alpha 3 (COL6A3), mRNA. /FEA=mRNA /GEN=COL6A3 /PROD=collagen, type VI, alpha 3 /DB_XREF=gi:4758027 /UG=Hs.80988 collagen, type VI, alpha 3 /FL=gb:NM_004369.1	201438_at
SPARC: secreted protein, acidic, cysteine-rich (osteonectin) (LOC6678) SEQ ID NOS: 56 (DNA) and 203 (amino acid)	gb:NM_003118.1 /DEF=Homo sapiens secreted protein, acidic, cysteine-rich (osteonectin) (SPARC), mRNA. /FEA=mRNA /GEN=SPARC /PROD=secreted protein, acidic, cysteine-rich(osteonectin) /DB_XREF=gi:4507170 /UG=Hs.111779 secreted protein, acidic, cysteine-rich (osteonectin) /FL=gb:BC004974.1 gb:J03040.1 gb:NM_003118.1	200665_s_at
TXNIP: thioredoxin interacting protein (LOC10628) SEQ ID NOS: 57 (DNA) and 204 (amino acid)	gb:NM_006472.1 /DEF=Homo sapiens upregulated by 1,25-dihydroxyvitamin D-3 (VDUP1), mRNA. /FEA=mRNA /GEN=VDUP1 /PROD=upregulated by 1,25-dihydroxyvitamin D-3 /DB_XREF=gi:5454161 /UG=Hs.179526 upregulated by 1,25-dihydroxyvitamin D-3 /FL=gb:NM_006472.1 gb:S73591.1	201010_s_at
MDK: midkine (neurite growth-promoting factor 2) (LOC4192) SEQ ID NOS: 58 (DNA) and 205 (amino acid)	gb:M69148.1 /DEF=Human midkine mRNA, complete cds. /FEA=mRNA /GEN=hMK-1 /PROD=midkine /DB_XREF=gi:182650 /UG=Hs.82045 midkine (neurite growth-promoting factor 2) /FL=gb:M69148.1 gb:NM_002391.1	209035_at
TXNRD1: thioredoxin reductase 1 (LOC7296) SEQ ID NOS: 59 (DNA) and 206 (amino acid)	gb:NM_003330.1 /DEF=Homo sapiens thioredoxin reductase 1 (TXNRD1), mRNA. /FEA=mRNA /GEN=TXNRD1 /PROD=thioredoxin reductase 1 /DB_XREF=gi:4507746 /UG=Hs.13046 thioredoxin reductase 1 /FL=gb:D88687.1 gb:AF077367.1 gb:NM_003330.1 gb:AF208018.1	201266_at
ARHD: ras homolog D (LOC29984) SEQ ID NOS: 60 (DNA) and 207 (amino acid)	gb:BC001338.1 /DEF=Homo sapiens, ras homolog gene family, member, clone MGC:5612, mRNA, complete cds. /FEA=mRNA /PROD=ras homolog gene family, member /DB_XREF=gi:12654980 /UG=Hs.15114 ras homolog gene family, member /FL=gb:BC001338.1 gb:NM_014578.1	209885_at

PSPHL: phosphoserine phosphatase-like (LOC8781) SEQ ID NOS: 61 (DNA) and 208 (amino acid)	gb:NM_003832.1 /DEF=Homo sapiens phosphoserine phosphatase-like (PSPHL), mRNA. /FEA=mRNA /GEN=PSPHL /PROD=L-3-phosphoserine phosphatase homolog /DB_XREF=gi:4502934 /UG=Hs.76845 phosphoserine phosphatase-like /FL=gb:NM_003832.1	205048_s_at
RAB25: RAB25 (LOC57111) SEQ ID NOS: 62 (DNA) and 209 (amino acid)	gb:NM_020387.1 /DEF=Homo sapiens CATX-8 protein (CATX-8), mRNA. /FEA=mRNA /GEN=CATX-8 /PROD=CATX-8 protein /DB_XREF=gi:9966860 /UG=Hs.150826 CATX-8 protein /FL=gb:AF083124.1 gb:NM_020387.1	218186_at
SPINT1: hepatocyte growth factor activator inhibitor 1 isoform 2 precursor (LOC6692) SEQ ID NOS: 63 (DNA) and 210 (amino acid)	gb:NM_003710.1 /DEF=Homo sapiens serine protease inhibitor, Kunitz type 1 (SPINT1), mRNA. /FEA=mRNA /GEN=SPINT1 /PROD=hepatocyte growth factor activator inhibitorprecursor /DB_XREF=gi:4504328 /UG=Hs.233950 serine protease inhibitor, Kunitz type 1 /FL=gb:BC004140.1 gb:AB000095.1 gb:NM_003710.1	202826_at
SPINT2: serine protease inhibitor, Kunitz type, 2 (LOC10653) SEQ ID NOS: 64 (DNA) and 211 (amino acid)	gb:AF027205.1 /DEF=Homo sapiens Kunitz-type protease inhibitor (kop) mRNA, complete cds. /FEA=mRNA /GEN=kop /PROD=Kunitz-type protease inhibitor /DB_XREF=gi:2598967 /UG=Hs.31439 serine protease inhibitor, Kunitz type, 2 /FL=gb:AF027205.1	210715_s_at
EMP3: epithelial membrane protein 3 (LOC2014) SEQ ID NOS: 65 (DNA) and 212 (amino acid)	gb:NM_001425.1 /DEF=Homo sapiens epithelial membrane protein 3 (EMP3), mRNA. /FEA=mRNA /GEN=EMP3 /PROD=epithelial membrane protein 3 /DB_XREF=gi:4503562 /UG=Hs.9999 epithelial membrane protein 3 /FL=gb:U52101.1 gb:U87947.1 gb:NM_001425.1	203729_at
TENS1: tensin-like SH2 domain-containing 1 (LOC64759) SEQ ID NOS: 66 (DNA) and 213 (amino acid)	gb:NM_022748.1 /DEF=Homo sapiens hypothetical protein FLJ13732 similar to tensin (FLJ13732), mRNA. /FEA=mRNA /GEN=FLJ13732 /PROD=hypothetical protein FLJ13732 similar to tensin /DB_XREF=gi:12232408 /UG=Hs.12210 hypothetical protein FLJ13732 similar to tensin /FL=gb:NM_022748.1	217853_at

HIF1A: hypoxia-inducible factor 1, alpha subunit isoform 1 (LOC3091) SEQ ID NOS: 67 (DNA) and 214 (amino acid)	gb:NM_001530.1 /DEF=Homo sapiens hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor) (HIF1A), mRNA. /FEA=mRNA /GEN=HIF1A /PROD=hypoxia-inducible factor 1, alpha subunit (basichelix-loop-helix transcription factor) /DB_XREF=gi:4504384 /UG=Hs.197540 hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor) /FL=gb:U29165.1 gb:AF304431.1 gb:NM_001530.1 gb:AF207601.1 gb:AF207602.1 gb:U22431.1	200989_at
ST14: matriptase (LOC6768) SEQ ID NOS: 68 (DNA) and 215 (amino acid)	gb:NM_021978.1 /DEF=Homo sapiens suppression of tumorigenicity 14 (colon carcinoma, matriptase, epithin) (ST14), mRNA. /FEA=mRNA /GEN=ST14 /PROD=suppression of tumorigenicity 14 (coloncarcinoma, matriptase, epithin) /DB_XREF=gi:11415039 /UG=Hs.56937 suppression of tumorigenicity 14 (colon carcinoma, matriptase, epithin) /FL=gb:AF057145.1 gb:NM_021978.1 gb:AB030036.1 gb:AF133086.1 gb:AF118224.2	202005_at
STK17A: serine/threonine kinase 17a (apoptosis-inducing) (LOC9263) SEQ ID NOS: 69 (DNA) and 216 (amino acid)	Consensus includes gb:AW194730 /FEA=EST /DB_XREF=gi:6473630 /DB_XREF=est:xn43d11.x1 /CLONE=IMAGE:2696469 /UG=Hs.9075 serinethreonine kinase 17a (apoptosis-inducing) /FL=gb:AB011420.1 gb:NM_004760.1	202693_s_at
SH3YL1: hypothetical protein DKFZP586F1318 (LOC26751) SEQ ID NOS: 70 (DNA) and 217 (amino acid)	gb:NM_015677.1 /DEF=Homo sapiens hypothetical protein (DKFZP586F1318), mRNA. /FEA=mRNA /GEN=DKFZP586F1318 /PROD=hypothetical protein /DB_XREF=gi:7661669 /UG=Hs.25213 hypothetical protein /FL=gb:NM_015677.1	204019_s_at
EXT1: exostoses (multiple) 1 (LOC2131) SEQ ID NOS: 71 (DNA) and 218 (amino acid)	gb:NM_000127.1 /DEF=Homo sapiens exostoses (multiple) 1 (EXT1), mRNA. /FEA=mRNA /GEN=EXT1 /PROD=exostoses (multiple) 1 /DB_XREF=gi:4557570 /UG=Hs.184161 exostoses (multiple) 1 /FL=gb:BC001174.1 gb:NM_000127.1	201995_at

GALNT7: polypeptide N-acetylgalactosaminyltransferase 7 (LOC51809) SEQ ID NOS: 72 (DNA) and 219 (amino acid)	gb:NM_017423.1 /DEF=Homo sapiens UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7 (GalNAc-T7) (GALNT7), mRNA. /FEA=mRNA /GEN=GALNT7 /PROD=polypeptide N-acetylgalactosaminyltransferase 7 /DB_XREF=gi:8393408 /UG=Hs.246315 UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7 (GalNAc-T7) /FL=gb:NM_017423.1	218313_s_at
SDC1: syndecan 1 (LOC6382) SEQ ID NOS: 73 (DNA) and 220 (amino acid)	gb:NM_002997.1 /DEF=Homo sapiens syndecan 1 (SDC1), mRNA. /FEA=mRNA /GEN=SDC1 /PROD=syndecan 1 /DB_XREF=gi:4506858 /UG=Hs.82109 syndecan 1 /FL=gb:J05392.1 gb:NM_002997.1	201287_s_at
ITGAV: integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51) (LOC3685) SEQ ID NOS: 74 (DNA) and 221 (amino acid)	Consensus includes gb:AI093579 /FEA=EST /DB_XREF=gi:3432555 /DB_XREF=est:qb15g06.x1 /CLONE=IMAGE:1696378 /UG=Hs.295726 integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51) /FL=gb:M14648.1 gb:NM_002210.1	202351_at
ANXA6: annexin VI isoform 1 (LOC309) SEQ ID NOS: 75 (DNA) and 222 (amino acid)	gb:NM_001155.2 /DEF=Homo sapiens annexin A6 (ANXA6), transcript variant 1, mRNA. /FEA=mRNA /GEN=ANXA6 /PROD=annexin VI isoform 1 /DB_XREF=gi:4809274 /UG=Hs.118796 annexin A6 /FL=gb:J03578.1 gb:D00510.1 gb:NM_001155.2	200982_s_at
PDGFC: platelet-derived growth factor C precursor (LOC56034) SEQ ID NOS: 76 (DNA) and 223 (amino acid)	gb:NM_016205.1 /DEF=Homo sapiens platelet derived growth factor C (PDGFC), mRNA. /FEA=mRNA /GEN=PDGFC /PROD=secretory growth factor-like protein fallotein /DB_XREF=gi:9994186 /UG=Hs.43080 platelet derived growth factor C /FL=gb:AF091434.1 gb:AF244813.1 gb:AB033831.1 gb:NM_016205.1	218718_at
FLNA: filamin 1 (actin-binding protein-280) (LOC2316) SEQ ID NOS: 77 (DNA) and 224 (amino acid)	Consensus includes gb:AI625550 /FEA=EST /DB_XREF=gi:4650481 /DB_XREF=est:ty57d06.x1 /CLONE=IMAGE:2283179 /UG=Hs.195464 filamin A, alpha (actin-binding protein-280)	214752_x_at

FLNA: filamin 1 (actin-binding protein-280) (LOC2316) SEQ ID NOS: 78 (DNA) and 225 (amino acid)	Consensus includes gb:AW051856 /FEA=EST /DB_XREF=gi:5914215 /DB_XREF=est:wz04a05.x1 /CLONE=IMAGE:2557040 /UG=Hs.195464 filamin A, alpha (actin-binding protein-280)	213746_s_at
TUBA3: tubulin, alpha 3 (LOC7846) SEQ ID NOS: 79 (DNA) and 226 (amino acid)	gb:AF141347.1 /DEF=Homo sapiens hum-a-tub2 alpha-tubulin mRNA, complete cds. /FEA=mRNA /PROD=alpha-tubulin /DB_XREF=gi:4929133 /UG=Hs.272897 Tubulin, alpha, brain-specific /FL=gb:AF141347.1 gb:NM_006009.1	209118_s_at
LOXL2: lysyl oxidase-like 2 (LOC4017) SEQ ID NOS: 80 (DNA) and 227 (amino acid)	gb:NM_002318.1 /DEF=Homo sapiens lysyl oxidase-like 2 (LOXL2), mRNA. /FEA=mRNA /GEN=LOXL2 /PROD=lysyl oxidase-like 2 /DB_XREF=gi:4505010 /UG=Hs.83354 lysyl oxidase-like 2 /FL=gb:BC000594.1 gb:U89942.1 gb:NM_002318.1 gb:AF1 17949.1	202998_s_at
CYR61: cysteine-rich, angiogenic inducer, 61 (LOC3491) SEQ ID NOS: 81 (DNA) and 228 (amino acid)	gb:AF003114.1 /DEF=Homo sapiens CYR61 mRNA, complete cds. /FEA=mRNA /GEN=CYR61 /DB_XREF=gi:6649848 /UG=Hs.8867 cysteine-rich, angiogenic inducer, 61 /FL=gb:AF003114.1	210764_s_at
GALNT3: polypeptide N-acetylgalactosaminyltransferase 3 (LOC2591) SEQ ID NOS: 82 (DNA) and 229 (amino acid)	Consensus includes gb:BF063271 /FEA=EST /DB_XREF=gi:10822181 /DB_XREF=est:7h87d05.x1 /CLONE=IMAGE:3322953 /UG=Hs.278611 UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3) /FL=gb:NM_004482.2	203397_s_at
MAP1B: microtubule-associated protein 1B isoform 1 (LOC4131) SEQ ID NOS: 83 (DNA) and 230 (amino acid)	Consensus includes gb:AL523076 /FEA=EST /DB_XREF=gi:12786569 /DB_XREF=est:AL523076 /CLONE=CS0DC001YI12 (3 prime) /UG=Hs.82503 H.sapiens mRNA for 3UTR of unknown protein	212233_at

TUBB-5: tubulin beta-5 (LOC84617) SEQ ID NOS: 84 (DNA) and 231 (amino acid)	gb:BC002654.1 /DEF=Homo sapiens, Similar to tubulin, beta, 4, clone MGC:4083, mRNA, complete cds. /FEA=mRNA /PROD=Similar to tubulin, beta, 4 /DB_XREF=gi:12803638 /UG=Hs.274398 Homo sapiens, Similar to tubulin, beta, 4, clone MGC:4083, mRNA, complete cds /FL=gb:BC002654.1	209191_at
TYMS: thymidylate synthetase (LOC7298) SEQ ID NOS: 85 (DNA) and 232 (amino acid)	gb:NM_001071.1 /DEF=Homo sapiens thymidylate synthetase (TYMS), mRNA. /FEA=mRNA /GEN=TYMS /PROD=thymidylate synthetase /DB_XREF=gi:4507750 /UG=Hs.82962 thymidylate synthetase /FL=gb:BC002567.1 gb:NM_001071.1	202589_at
IFI16: interferon, gamma-inducible protein 16 (LOC3428) SEQ ID NOS: 86 (DNA) and 233 (amino acid)	gb:NM_005531.1 /DEF=Homo sapiens interferon, gamma-inducible protein 16 (IFI16), mRNA. /FEA=mRNA /GEN=IFI16 /PROD=interferon, gamma-inducible protein 16 /DB_XREF=gi:5031778 /UG=Hs.155530 interferon, gamma-inducible protein 16 /FL=gb:M63838.1 gb:NM_005531.1	206332_s_at
GRB10: growth factor receptor-bound protein 10 (LOC2887) SEQ ID NOS: 87 (DNA) and 234 (amino acid)	gb:D86962.1 /DEF=Human mRNA for KIAA0207 gene, complete cds. /FEA=mRNA /GEN=KIAA0207 /DB_XREF=gi:1503997 /UG=Hs.81875 growth factor receptor-bound protein 10 /FL=gb:D86962.1 gb:AF000017.1	209409_at
FLNA: filamin 1 (actin-binding protein-280) (LOC2316) SEQ ID NOS: 88 (DNA) and 235 (amino acid)	gb:NM_001456.1 /DEF=Homo sapiens filamin A, alpha (actin-binding protein-280) (FLNA), mRNA. /FEA=mRNA /GEN=FLNA /PROD=filamin 1 (actin-binding protein-280) /DB_XREF=gi:4503744 /UG=Hs.195464 filamin A, alpha (actin-binding protein-280) /FL=gb:NM_001456.1	200859_x_at
TNC: tenascin C (hexabrachion) (LOC3371) SEQ ID NOS: 89 (DNA) and 236 (amino acid)	gb:NM_002160.1 /DEF=Homo sapiens hexabrachion (tenascin C, cytotactin) (HXB), mRNA. /FEA=mRNA /GEN=HXB /PROD=hexabrachion (tenascin C, cytotactin) /DB_XREF=gi:4504548 /UG=Hs.289114 hexabrachion (tenascin C, cytotactin) /FL=gb:M55618.1 gb:NM_002160.1	201645_at

SLC26A2: sulfate anion transporter 1 (LOC1836) SEQ ID NOS: 90 (DNA) and 237 (amino acid)	Consensus includes gb:AI025519 /FEA=EST /DB_XREF=gi:3241132 /DB_XREF=est:ov75c04.x1 /CLONE=IMAGE:1643142 /UG=Hs.29981 solute carrier family 26 (sulfate transporter), member 2 /FL=gb:NM_000112.1 gb:U14528.1	205097_at
KIAA0746: KIAA0746 protein (LOC23231) SEQ ID NOS: 91 (DNA) and 238 (amino acid)	Consensus includes gb:AB018289.1 /DEF=Homo sapiens mRNA for KIAA0746 protein, partial cds. /FEA=mRNA /GEN=KIAA0746 /PROD=KIAA0746 protein /DB_XREF=gi:3882212 /UG=Hs.49500 KIAA0746 protein	212314_at
LAMP1 : lysosomal-associated membrane protein 1 (LOC3916) SEQ ID NOS: 92 (DNA) and 239 (amino acid)	gb:NM_005561.2 /DEF=Homo sapiens lysosomal-associated membrane protein 1 (LAMP1), mRNA. /FEA=mRNA /GEN=LAMP1 /PROD=lysosomal-associated membrane protein 1 /DB_XREF=gi:7669500 /UG=Hs.150101 lysosomal-associated membrane protein 1 /FL=gb:J04182.1 gb:J03263.1 gb:NM_005561.2	201553_s_at
DPYSL2: dihydropyrimidinase-like 2 (LOC1808) SEQ ID NOS: 93 (DNA) and 240 (amino acid)	gb:NM_001386.1 /DEF=Homo sapiens dihydropyrimidinase-like 2 (DPYSL2), mRNA. /FEA=mRNA /GEN=DPYSL2 /PROD=dihydropyrimidinase-like 2 /DB_XREF=gi:4503376 /UG=Hs.173381 dihydropyrimidinase-like 2 /FL=gb:U17279.1 gb:D78013.1 gb:U97105.1 gb:NM_001386.1	200762_at
IFI16: interferon, gamma-inducible protein 16 (LOC3428) SEQ ID NOS: 94 (DNA) and 241 (amino acid)	gb:AF208043.1 /DEF=Homo sapiens IFI16b (IFI16b) mRNA, complete cds. /FEA=mRNA /GEN=IFI16b /PROD=IFI16b /DB_XREF=gi:6644296 /UG=Hs.155530 interferon, gamma-inducible protein 16 /FL=gb:AF208043.1	208966_x_at
KPNB2: karyopherin beta 2 (LOC3842) SEQ ID NOS: 95 (DNA) and 242 (amino acid)	Consensus includes gb:AI307759 /FEA=EST /DB_XREF=gi:4002363 /DB_XREF=est:tb24g08.x1 /CLONE=IMAGE:2055326 /UG=Hs.168075 karyopherin (importin) beta 2	221829_s_at

PRNP: prion protein preproprotein (LOC5621) SEQ ID NOS: 96 (DNA) and 243 (amino acid)	gb:NM_000311.1 /DEF=Homo sapiens prion protein (p27-30) (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia) (PRNP), mRNA. /FEA=mRNA /GEN=PRNP /PROD=prion protein /DB_XREF=gi:4506112 /UG=Hs.74621 prion protein (p27-30) (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia) /FL=gb:AY008282.1 gb:M13899.1 gb:NM_000311.1	201300_s_at
RAI14: retinoic acid induced 14 (LOC26064) SEQ ID NOS: 97 (DNA) and 244 (amino acid)	gb:NM_015577.1 /DEF=Homo sapiens novel retinal pigment epithelial gene (NORPEG), mRNA. /FEA=mRNA /GEN=NORPEG /PROD=DKFZP564G013 protein /DB_XREF=gi:13470085 /UG=Hs.15165 novel retinal pigment epithelial gene /FL=gb:NM_015577.1 gb:AF155135.1	202052_s_at
JAG1: jagged 1 precursor (LOC182) SEQ ID NOS: 98 (DNA) and 245 (amino acid)	gb:U61276.1 /DEF=Human transmembrane protein Jagged 1 (HJ1) mRNA, complete cds. /FEA=mRNA /GEN=HJ1 /PROD=transmembrane protein Jagged 1 /DB_XREF=gi:1438936 /UG=Hs.91143 jagged 1 (Alagille syndrome) /FL=gb:U61276.1 gb:U73936.1 gb:AF003837.1 gb:AF028593.1 gb:NM_000214.1	209098_s_at
CLIC4: chloride intracellular channel 4 (LOC25932) SEQ ID NOS: 99 (DNA) and 246 (amino acid)	gb:NM_013943.1 /DEF=Homo sapiens chloride intracellular channel 4 (CLIC4), mRNA. /FEA=mRNA /GEN=CLIC4 /PROD=chloride intracellular channel 4 /DB_XREF=gi:7330334 /UG=Hs.25035 chloride intracellular channel 4 /FL=gb:AF109196.1 gb:AF097330.1 gb:AL117424.1 gb:NM_013943.1	201560_at
TP53I3: tumor protein p53 inducible protein 3 (LOC9540) SEQ ID NOS: 100 (DNA) and 247 (amino acid)	gb:BC000474.1 /DEF=Homo sapiens, quinone oxidoreductase homolog, clone MGC:8642, mRNA, complete cds. /FEA=mRNA /PROD=quinone oxidoreductase homolog /DB_XREF=gi:12653408 /UG=Hs.50649 quinone oxidoreductase homolog /FL=gb:BC000474.1	210609_s_at
EFA6R: ADP-ribosylation factor guanine nucleotide factor 6 (LOC23362) SEQ ID NOS: 101 (DNA) and 248 (amino acid)	Consensus includes gb:AW117368 /FEA=EST /DB_XREF=gi:6085952 /DB_XREF=est:xd88h01.x1 /CLONE=IMAGE:2604721 /UG=Hs.6763 KIAA0942 protein /FL=gb:AF243495.2 gb:NM_015310.1	203354_s_at

JUP: junction plakoglobin (LOC3728) SEQ ID NOS: 102 (DNA) and 249 (amino acid)	gb:NM_021991.1 /DEF=Homo sapiens junction plakoglobin (JUP), transcript variant 2, mRNA. /FEA=mRNA /GEN=JUP /PROD=junction plakoglobin, isoform 1 /DB_XREF=gi:12056467 /UG=Hs.2340 junction plakoglobin /FL=gb:NM_021991.1 gb:BC000441.1	201015_s_at
PAPSS2: 3'-phosphoadenosine 5'-phosphosulfate synthase 2 (LOC9060) SEQ ID NOS: 103 (DNA) and 250 (amino acid)	gb:NM_004670.1 /DEF=Homo sapiens 3-phosphoadenosine 5-phosphosulfate synthase 2 (PAPSS2), mRNA. /FEA=mRNA /GEN=PAPSS2 /PROD=3-prime-phosphoadenosine 5-prime-phosphosulfate synthase 2 /DB_XREF=gi:4758879 /UG=Hs.274230 3-phosphoadenosine 5-phosphosulfate synthase 2 /FL=gb:AF150754.2 gb:AF313907.1 gb:AF091242.1 gb:NM_004670.1 gb:AF074331.1 gb:AF173365.1	203059_s_at
DKK3: dickkopf homolog 3 (LOC27122) SEQ ID NOS: 104 (DNA) and 251 (amino acid)	Consensus includes gb:AU148057 /FEA=EST /DB_XREF=gi:11009578 /DB_XREF=est:AU148057 /CLONE=MAMMA1002489 /UG=Hs.278503 regulated in glioma	214247_s_at
JAG1: jagged 1 precursor (LOC182) SEQ ID NOS: 105 (DNA) and 252 (amino acid)	Consensus includes gb:U77914.1 /DEF=Human soluble protein Jagged mRNA, partial cds. /FEA=mRNA /PROD=soluble protein Jagged /DB_XREF=gi:1684889 /UG=Hs.91143 jagged 1 (Alagille syndrome)	216268_s_at
CALD1: caldesmon 1 isoform 3 (LOC800) SEQ ID NOS: 106 (DNA) and 253 (amino acid)	Consensus includes gb:AL583520 /FEA=EST /DB_XREF=gi:12952562 /DB_XREF=est:AL583520 /CLONE=CS0DC024YE13 (5 prime) /UG=Hs.182183 Homo sapiens mRNA for caldesmon, 3 UTR	212077_at
DPYSL3: dihydropyrimidinase-like 3 (LOC1809) SEQ ID NOS: 107 (DNA) and 254 (amino acid)	Consensus includes gb:W72516 /FEA=EST /DB_XREF=gi:1382173 /DB_XREF=est:zd64g05.s1 /CLONE=IMAGE:345464 /UG=Hs.74566 dihydropyrimidinase-like 3 /FL=gb:D78014.1 gb:NM_001387.1	201430_s_at

PMP22: peripheral myelin protein 22 (LOC5376) SEQ ID NOS: 108 (DNA) and 255 (amino acid)	gb:L03203.1 /DEF=Human peripheral myelin protein 22 (GAS3) mRNA, complete cds. /FEA=mRNA /GEN=GAS3 /PROD=peripheral myelin protein 22 /DB_XREF=gi:182984 /UG=Hs.103724 peripheral myelin protein 22 /FL=gb:L03203.1	210139_s_at
ALCAM: activated leukocyte cell adhesion molecule (LOC214) SEQ ID NOS: 109 (DNA) and 256 (amino acid)	Consensus includes gb:BF242905 /FEA=EST /DB_XREF=gi:11156833 /DB_XREF=est:601877949F1 /CLONE=IMAGE:4106028 /UG=Hs.10247 activated leucocyte cell adhesion molecule /FL=gb:NM_001627.1 gb:L38608.1	201951_at
PAPSS2: 3'-phosphoadenosine 5'-phosphosulfate synthase 2 (LOC9060) SEQ ID NOS: 110 (DNA) and 257 (amino acid)	Consensus includes gb:AW299958 /FEA=EST /DB_XREF=gi:6709635 /DB_XREF=est:xs44g05.x1 /CLONE=IMAGE:2772536 /UG=Hs.274230 3-phosphoadenosine 5-phosphosulfate synthase 2 /FL=gb:AF150754.2 gb:AF313907.1 gb:AF091242.1 gb:NM_004670.1 gb:AF074331.1 gb:AF173365.1	203058_s_at
KPNB2: karyopherin beta 2 (LOC3842) SEQ ID NOS: 111 (DNA) and 258 (amino acid)	gb:NM_002270.1 /DEF=Homo sapiens karyopherin (importin) beta 2 (KPNB2), mRNA. /FEA=mRNA /GEN=KPNB2 /PROD=karyopherin (importin) beta 2 /DB_XREF=gi:4504906 /UG=Hs.168075 karyopherin (importin) beta 2 /FL=gb:U70322.1 gb:NM_002270.1	207657_x_at
PTPRE: protein tyrosine phosphatase, receptor type, E isoform 1 precursor (LOC5791) SEQ ID NOS: 112 (DNA) and 259 (amino acid)	Consensus includes gb:AA775177 /FEA=EST /DB_XREF=gi:2834511 /DB_XREF=est:ac79a06.s1 /CLONE=IMAGE:868786 /UG=Hs.31137 protein tyrosine phosphatase, receptor type, E /FL=gb:NM_006504.1	221840_at
TRB2: tribbles homolog 2 (LOC28951) SEQ ID NOS: 113 (DNA) and 260 (amino acid)	gb:NM_021643.1 /DEF=Homo sapiens GS3955 protein (GS3955), mRNA. /FEA=mRNA /GEN=GS3955 /PROD=GS3955 protein /DB_XREF=gi:11056053 /UG=Hs.155418 GS3955 protein /FL=gb:NM_021643.1 gb:BC002637.1 gb:D87119.1	202478_at

COL13A1: alpha 1 type XIII collagen isoform 1 (LOC130) SEQ ID NOS: 114 (DNA) and 261 (amino acid)	gb:M33653.1 /DEF=Human (clones HT-125,133) alpha-2 type IV collagen (COL4A2) mRNA, complete cds. /FEA=mRNA /GEN=COL4A2 /PROD=alpha-2 type IV collagen /DB_XREF=gi:180828 /UG=Hs.211933 collagen, type XIII, alpha 1 /FL=gb:M33653.1	211343_s_at
PALM2: paralemmin 2 (LOC114299) SEQ ID NOS: 115 (DNA) and 262 (amino acid)	gb:NM_007203.1 /DEF=Homo sapiens A kinase (PRKA) anchor protein 2 (AKAP2), mRNA. /FEA=mRNA /GEN=AKAP2 /PROD=A kinase (PRKA) anchor protein 2 /DB_XREF=gi:6005708 /UG=Hs.42322 A kinase (PRKA) anchor protein 2 /FL=gb:AB023137.1 gb:NM_007203.1	202760_s_at
GJA1: connexin 43 (LOC2697) SEQ ID NOS: 116 (DNA) and 263 (amino acid)	gb:NM_000165.2 /DEF=Homo sapiens gap junction protein, alpha 1, 43kD (connexin 43) (GJA1), mRNA. /FEA=mRNA /GEN=GJA1 /PROD=connexin 43 /DB_XREF=gi:4755136 /UG=Hs.74471 gap junction protein, alpha 1, 43kD (connexin 43) /FL=gb:M65188.1 gb:NM_000165.2	201667_at
FLJ10901: hypothetical protein FLJ10901 (LOC55765) SEQ ID NOS: 117 (DNA) and 264 (amino acid)	gb:NM_018265.1 /DEF=Homo sapiens hypothetical protein FLJ10901 (FLJ10901), mRNA. /FEA=mRNA /GEN=FLJ10901 /PROD=hypothetical protein FLJ10901 /DB_XREF=gi:8922753 /UG=Hs.73239 hypothetical protein FLJ10901 /FL=gb:NM_018265.1	219010_at
EFEMP1: EGF-containing fibulin-like extracellular matrix protein 1 isoform a precursor (LOC2202) SEQ ID NOS: 118 (DNA) and 265 (amino acid)	Consensus includes gb:AI826799 /FEA=EST /DB_XREF=gi:5447470 /DB_XREF=est:wk56d07.x1 /CLONE=IMAGE:2419405 /UG=Hs.76224 EGF-containing fibulin-like extracellular matrix protein 1 /FL=gb:U03877. 1 gb:NM_004105.2	201842_s_at
NRP1: neuropilin 1 (LOC8829) SEQ ID NOS: 119 (DNA) and 266 (amino acid)	Consensus includes gb:BE620457 /FEA=EST /DB_XREF=gi:9891395 /DB_XREF=est:601483690F1 /CLONE=IMAGE:3886055 /UG=Hs.69285 neuropilin 1 /FL=gb:AF01 8956.1 gb:AF016050.1 gb:NM_0O3873.1	212298_at

CLDN7: claudin 7 (LOC1366) SEQ ID NOS: 120 (DNA) and 267 (amino acid)	gb:NM_001307.1 /DEF=Homo sapiens claudin 7 (CLDN7), mRNA. /FEA=mRNA /GEN=CLDN7 /PROD=claudin 7 /DB_XREF=gi:10835007 /UG=Hs.278562 claudin 7 /FL=gb:NM_001307.1 gb:BC001055.1	202790_at
CED-6: PTB domain adaptor protein CED-6 (LOC51454) SEQ ID NOS: 121 (DNA) and 268 (amino acid)	gb:NM_016315.1 /DEF=Homo sapiens CED-6 protein (CED-6), mRNA. /FEA=mRNA /GEN=CED-6 /PROD=CED-6 protein /DB_XREF=gi:7705317 /UG=Hs.107056 CED-6 protein /FL=gb:AF200715. 1 gb:AF191771.1 gb:NM_016315.1	204237_at
CSPG2: chondroitin sulfate proteoglycan 2 (versican) (LOC1462) SEQ ID NOS: 122 (DNA) and 269 (amino acid)	Consensus includes gb:BF590263 /FEA=EST /DB_XREF=gi:11682587 /DB_XREF=est:nab22b12.x1 /CLONE=IMAGE:3266638 /UG=Hs.81800 chondroitin sulfate proteoglycan 2 (versican) /FL=gb:NM_004385.1	204619_s_at
KPNB2: karyopherin beta 2 (LOC3842) SEQ ID NOS: 123 (DNA) and 270 (amino acid)	gb:U72069.1 /DEF=Human karyopherin beta2 mRNA, complete cds. /FEA=mRNA /PROD=karyopherin beta2 /DB_XREF=gi:1657775 /UG=Hs.168075 karyopherin (importin) beta 2 /FL=gb:U72069.1 gb:U72395.1	209226_s_at
MLAT4: myxoid liposarcoma associated protein 4 (LOC55214) SEQ ID NOS: 124 (DNA) and 271 (amino acid)	gb:NM_018192.1 /DEF=Homo sapiens hypothetical protein FLJ10718 (FLJ10718), mRNA. /FEA=mRNA /GEN=FLJ10718 /PROD=hypothetical protein FLJ10718 /DB_XREF=gi:8922618 /UG=Hs.42824 hypothetical protein FLJ10718 /FL=gb:NM_018192.1	218717_s_at
TPM1: tropomyosin 1 (alpha) (LOC7168) SEQ ID NOS: 125 (DNA) and 272 (amino acid)	gb:Z24727.1 /DEF=H.sapiens tropomyosin isoform mRNA, complete CDS. /FEA=mRNA /PROD=tropomyosin isoform /DB_XREF=gi:854188 /UG=Hs.77899 tropomyosin 1 (alpha) /FL=gb:Z24727.1	210986_s_at
LY96: MD-2 protein (LOC23643) SEQ ID NOS: 126 (DNA) and 273 (amino acid)	gb:NM_015364.1 /DEF=Homo sapiens MD-2 protein (MD-2), mRNA. /FEA=mRNA /GEN=MD-2 /PROD=MD-2 protein /DB_XREF=gi:7662503 /UG=Hs.69328 MD-2 protein /FL=gb:AB018549.1 gb:NM_015364.1 gb:AF168121.1	206584_at

COL6A1: collagen, type VI, alpha 1 precursor (LOC1291) SEQ ID NOS: 127 (DNA) and 274 (amino acid)	Consensus includes gb:AI141603 /FEA=EST /DB_XREF=gi:3649060 /DB_XREF=est:qa90h10.x1 /CLONE=IMAGE:1694083 /UG=Hs.108885 collagen, type VI, alpha 1	212091_s_at
CDC42EP3: Cdc42 effector protein 3 (LOC10602) SEQ ID NOS: 128 (DNA) and 275 (amino acid)	gb:AL136842.1 /DEF=Homo sapiens mRNA; cDNA DKFZp434A0530 (from clone DKFZp434A0530); complete cds. /FEA=mRNA /GEN=DKFZp434A0530 /PROD=hypothetical protein /DB_XREF=gi:6807668 /UG=Hs.260024 Cdc42 effector protein 3 /FL=gb:AF094521.1 gb:AF104857.1 gb:NM_006449.1 gb:AF164118.1 gb:AL136842.1	209288_s_at
JTB: jumping translocation breakpoint (LOC10899) SEQ ID NOS: 129 (DNA) and 276 (amino acid)	gb:NM_006694.1 /DEF=Homo sapiens jumping translocation breakpoint (JTB), mRNA. /FEA=mRNA /GEN=JTB /PROD=jumping translocation breakpoint /DB_XREF=gi:5729888 /UG=Hs.6396 jumping translocation breakpoint /FL=gb:BC000499.1 gb:BC001363.1 gb:BC000996.2 gb:BC001667.1 gb:AB016488.1 gb:AF131797.1 gb:NM_006694.1 gb:AF115850.2	200048_s_at
CDH2: cadherin 2, type 1 preproprotein (LOC1000) SEQ ID NOS: 130 (DNA) and 277 (amino acid)	gb:M34064.1 /DEF=Human N-cadherin mRNA, complete cds. /FEA=mRNA /GEN=NCAD /DB_XREF=gi:416292 /UG=Hs.161 cadherin 2, type 1, N-cadherin (neuronal) /FL=gb:M34064.1 gb:NM_001792.1	203440_at
MYLK: myosin light chain kinase isoform 6 (LOC4638) SEQ ID NOS: 131 (DNA) and 278 (amino acid)	gb:NM_005965.1 /DEF=Homo sapiens myosin, light polypeptide kinase (MYLK), mRNA. /FEA=mRNA /GEN=MYLK /PROD=myosin, light polypeptide kinase /DB_XREF=gi:5174600 /UG=Hs.211582 myosin, light polypeptide kinase /FL=gb:AB037663.1 gb:NM_005965.1 gb:AF069601.2	202555_s_at

COL4A1: alpha 1 type IV collagen preproprotein (LOC1282) SEQ ID NOS: 132 (DNA) and 279 (amino acid)	Consensus includes gb:NM_001845.1 /DEF=Homo sapiens collagen, type IV, alpha 1 (COL4A1), mRNA. /FEA=CDS /GEN=COL4A1 /PROD=collagen, type IV, alpha 1 /DB_XREF=gi:7656984 /UG=Hs.119129 collagen, type IV, alpha 1 /FL=gb:NM_001845.1	211981_at
PROS1: protein S (alpha) (LOC5627) SEQ ID NOS: 133 (DNA) and 280 (amino acid)	gb:NM_000313.1 /DEF=Homo sapiens protein S (alpha) (PROS1), mRNA. /FEA=mRNA /GEN=PROS1 /PROD=protein S (alpha) /DB_XREF=gi:4506116 /UG=Hs.64016 protein S (alpha) /FL=gb:M15036.1 gb:NM_000313.1	207808_s_at
EFEMP1: EGF-containing fibulin-like extracellular matrix protein 1 isoform a precursor (LOC2202) SEQ ID NOS: 134 (DNA) and 281 (amino acid)	gb:NM_004105.2 /DEF=Homo sapiens EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1), transcript variant 1, mRNA. /FEA=mRNA /GEN=EFEMP1 /PROD=EGF-containing fibulin-like extracellular matrixprotein 1 precursor, isoform a precursor /DB_XREF=gi:9665261 /UG=Hs.76224 EGF-containing fibulin-like extracellular matrix protein 1 /FL=gb:U03877.1 gb:NM_004105.2	201843_s_at
CCL2: small inducible cytokine A2 precursor (LOC6347) SEQ ID NOS: 135 (DNA) and 282 (amino acid)	Consensus includes gb:S69738.1 /DEF=MCP-1=monocyte chemotactic protein human, aortic endothelial cells, mRNA, 661 nt. /FEA=mRNA /GEN=MCP-1 /PROD=MCP-1 /DB_XREF=gi:545464 /UG=Hs.303649 small inducible cytokine A2 (monocyte chemotactic protein 1, homologous to mouse Sig-je)	216598_s_at
DFNA5: deafness, autosomal dominant 5 protein (LOC1687) SEQ ID NOS: 136 (DNA) and 283 (amino acid)	gb:NM_004403.1 /DEF=Homo sapiens deafness, autosomal dominant 5 (DFNA5), mRNA. /FEA=mRNA /GEN=DFNA5 /PROD=deafness, autosomal dominant 5 protein /DB_XREF=gi:4758153 /UG=Hs.13530 deafness, autosomal dominant 5 /FL=gb:AF073308.1 gb:NM_004403.1 gb:AF007790.2	203695_s_at
TPM1: tropomyosin 1 (alpha) (LOC7168) SEQ ID NOS: 137 (DNA) and 284 (amino acid)	gb:M19267.1 /DEF=Human tropomyosin mRNA, complete cds. /FEA=mRNA /DB_XREF=gi:339943 /UG=Hs.77899 tropomyosin 1 (alpha) /FL=gb:M19267.1	210987_x_at

DDAH1: dimethylarginine dimethylaminohydrolase 1 (LOC23576) SEQ ID NOS: 138 (DNA) and 285 (amino acid)	Consensus includes gb:AL078459 /DEF=Human DNA sequence from clone RP4-621F18 on chromosome 1p11.4-21.3. Contains the 3 end of the gene for ng,ng dimethylarginine dimethylaminohydrolase (EC 3.5.3.18), ESTs, STSs and GSSs /FEA=mRNA /DB_XREF=gi:5791502 /UG=Hs.303180 dimethylarginine dimethylaminohydrolase 1 /FL=gb:AB001915.1 gb:NM_012137.1	209094_at
PMAIP1: phorbol-12-myristate-13-acetate-induced protein 1 (LOC5366) SEQ ID NOS: 139 (DNA) and 286 (amino acid)	Consensus includes gb:AI857639 /FEA=EST /DB_XREF=gi:5511255 /DB_XREF=est:wk95g09.x1 /CLONE=IMAGE:2423200 /UG=Hs.96 phorbol-12-myristate-13-acetate-induced protein 1 /FL=gb:NM_021127.1	204285_s_at
ACOX2: acyl-Coenzyme A oxidase 2, branched chain (LOC8309) SEQ ID NOS: 140 (DNA) and 287 (amino acid)	gb:NM_003500.1 /DEF=Homo sapiens acyl-Coenzyme A oxidase 2, branched chain (ACOX2), mRNA. /FEA=mRNA /GEN=ACOX2 /PROD=acyl-Coenzyme A oxidase 2, branched chain /DB_XREF=gi:4501868 /UG=Hs.9795 acyl-Coenzyme A oxidase 2, branched chain /FL=gb:NM_003500.1	205364_at
GDI1: GDP dissociation inhibitor 1 (LOC2664) SEQ ID NOS: 141 (DNA) and 288 (amino acid)	gb:NM_001493.1 /DEF=Homo sapiens GDP dissociation inhibitor 1 (GDI1), mRNA. /FEA=mRNA /GEN=GDI1 /PROD=GDP dissociation inhibitor 1 /DB_XREF=gi:4503970 /UG=Hs.74576 GDP dissociation inhibitor 1 /FL=gb:BC000317.1 gb:NM_001493.1 gb:D45021.1	201864_at
DPYSL3: dihydropyrimidinase-like 3 (LOC1809) SEQ ID NOS: 142 (DNA) and 289 (amino acid)	gb:NM_001387.1 /DEF=Homo sapiens dihydropyrimidinase-like 3 (DPYSL3), mRNA. /FEA=mRNA /GEN=DPYSL3 /PROD=dihydropyrimidinase-like 3 /DB_XREF=gi:4503378 /UG=Hs.74566 dihydropyrimidinase-like 3 /FL=gb:D78014.1 gb:NM_001387.1	201431_s_at
APOC1: apolipoprotein C-I precursor (LOC341) SEQ ID NOS: 143 (DNA) and 290 (amino acid)	Consensus includes gb:W79394 /FEA=EST /DB_XREF=gi:1390665 /DB_XREF=est:zd80c07.s1 /CLONE=IMAGE:346956 /UG=Hs.268571 apolipoprotein C-I	213553_x_at

TTC3: tetratricopeptide repeat domain 3 (LOC7267) SEQ ID NOS: 144 (DNA) and 291 (amino acid)	gb:NM_003316.1 /DEF=Homo sapiens tetratricopeptide repeat domain 3 (TTC3), mRNA. /FEA=mRNA /GEN=TTC3 /PROD=tetratricopeptide repeat domain 3 /DB_XREF=gi:10835036 /UG=Hs.118174 tetratricopeptide repeat domain 3 /FL=gb:NM_003316.1 gb:D84295.1	208073_x_at
SNX6: sorting nexin 6 isoform a (LOC58533) SEQ ID NOS: 145 (DNA) and 292 (amino acid)	gb:NM_021249.1 /DEF=Homo sapiens sorting nexin 6 (SNX6), mRNA. /FEA=mRNA /GEN=SNX6 /PROD=sorting nexin 6 /DB_XREF=gi:13027619 /UG=Hs.284291 sorting nexin 6 /FL=gb:BC001798.1 gb:NM_021249.1 gb:AF121856.1	217789_at
CKAP4: transmembrane protein (63kD), endoplasmic reticulum/Golgi interm (LOC10970) SEQ ID NOS: 146 (DNA) and 293 (amino acid)	Consensus includes gb:AW029619 /FEA=EST /DB_XREF=gi:5888375 /DB_XREF=est:wx14e05.x1 /CLONE=IMAGE:2543648 /UG=Hs.74368 transmembrane protein (63kD), endoplasmic reticulumGolgi intermediate compartment /FL=gb:NM_006825.1	200998_s_at
TUBB: tubulin, beta polypeptide (LOC7280) SEQ ID NOS: 147 (DNA) and 294 (amino acid)	gb:NM_001069.1 /DEF=Homo sapiens tubulin, beta polypeptide (TUBB), mRNA. /FEA=mRNA /GEN=TUBB /PROD=tubulin, beta polypeptide /DB_XREF=gi:4507728 /UG=Hs.179661 tubulin, beta polypeptide /FL=gb:BC001194.1 gb:NM_001069.1	204141_at

The biomarkers provided in Table 1, which include the nucleotide sequences of SEQ ID NOS:1-147 and the amino acid sequences of SEQ ID NOS:148-294, referred to herein as a total of 147 biomarkers with reference to the Unigene Title, includes 40 cases where multiple probe sets measure the intensity of a single biomarker (at most, three probe sets for one biomarker). In these cases, the redundant probe sets reference the same full-length cDNA and protein sequences. Table 2 provides a correlation between the NCBI locus IDs and the probe set IDs.

TABLE 2 - Correlation between NCBI Locus IDs and Probe Set IDs

NCBI Locus ID	Number of Probe sets	Probe set IDs
182	3	209099_x_at, 209098_s_at, 216268_s_at
1462	3	204620_s_at, 221731_x_at, 204619_s_at
2316	3	214752_x_at, 213746_s_at, 200859_x_at
3842	3	221829_s_at, 207657_x_at, 209226_s_at
9060	3	203060_s_at, 203059_s_at, 203058_s_at
10899	3	210434_x_at, 210927_x_at, 200048_s_at
214	2	201952_at, 201951_at
1291	2	213428_s_at, 212091_s_at
1508	2	200839_s_at, 200838_at
1809	2	201430_s_at, 201431_s_at
2202	2	201842_s_at, 201843_s_at
2810	2	33322_i_at, 33323_r_at
3428	2	206332_s_at, 208966_x_at
3491	2	201289_at, 210764_s_at
7168	2	210986_s_at, 210987_x_at
8781	2	212509_s_at, 205048_s_at
10628	2	201008_s_at, 201010_s_at
130	1	211343_s_at
309	1	200982_s_at
341	1	213553_x_at
754	1	200677_at
800	1	212077_at
999	1	201131_s_at
1000	1	203440_at
1075	1	201487_at
1282	1	211981_at
1292	1	209156_s_at
1293	1	201438_at
1366	1	202790_at
1490	1	209101_at
1687	1	203695_s_at
1808	1	200762_at
1836	1	205097_at
2014	1	203729_at
2115	1	221911_at
2131	1	201995_at
2150	1	213506_at
2273	1	201540_at
2591	1	203397_s_at
2664	1	201864_at
2697	1	201667_at
2791	1	204115_at
2887	1	209409_at

3091	1	200989_at
3371	1	201645_at
3383	1	202637_s_at
3576	1	211506_s_at
3685	1	202351_at
3728	1	201015_s_at
3855	1	209016_s_at
3856	1	209008_x_at
3875	1	201596_x_at
3880	1	201650_at
3916	1	201553_s_at
4017	1	202998_s_at
4131	1	212233_at
4192	1	209035_at
4638	1	202555_s_at
5066	1	202336_s_at
5270	1	212190_at
5366	1	204285_s_at
5376	1	210139_s_at
5441	1	211730_s_at
5621	1	201300_s_at
5627	1	207808_s_at
5791	1	221840_at
5834	1	201481_s_at
6137	1	212191_x_at
6159	1	213969_x_at
6280	1	203535_at
6303	1	210592_s_at
6347	1	216598_s_at
6382	1	201287_s_at
6535	1	202219_at
6678	1	200665_s_at
6692	1	202826_at
6748	1	201004_at
6768	1	202005_at
6772	1	200887_s_at
7045	1	201506_at
7267	1	208073_x_at
7280	1	204141_at
7296	1	201266_at
7298	1	202589_at
7345	1	201387_s_at
7358	1	203343_at
7846	1	209118_s_at
8309	1	205364_at
8829	1	212298_at
9263	1	202693_s_at

9540	1	210609_s_at
10135	1	217738_at
10602	1	209288_s_at
10653	1	210715_s_at
10962	1	211071_s_at
10970	1	200998_s_at
11098	1	202458_at
11199	1	210143_at
22943	1	204602_at
23231	1	212314_at
23362	1	203354_s_at
23576	1	209094_at
23643	1	206584_at
25932	1	201560_at
26064	1	202052_s_at
26751	1	204019_s_at
27122	1	214247_s_at
28951	1	202478_at
29984	1	209885_at
51065	1	218007_s_at
51454	1	204237_at
51809	1	218313_s_at
54407	1	218041_x_at
55214	1	218717_s_at
55765	1	219010_at
56034	1	218718_at
57111	1	218186_at
57402	1	218677_at
58533	1	217789_at
64759	1	217853_at
84617	1	209191_at
113146	1	212992_at
114299	1	202760_s_at
347902	1	222108_at

The biomarkers have expression levels in the cells that may be dependent on the activity of the EGFR signal transduction pathway, and that are also highly correlated with EGFR modulator sensitivity exhibited by the cells. Biomarkers serve as useful molecular tools for predicting a response to EGFR modulators, preferably biological molecules, small molecules, and the like that affect EGFR kinase activity via direct or indirect inhibition or antagonism of EGFR kinase function or activity.

EGFR MODULATORS

As used herein, the term "EGFR modulator" is intended to mean a compound or drug that is a biological molecule or a small molecule that directly or indirectly modulates EGFR activity or the EGFR signal transduction pathway. Thus, compounds or drugs as used herein is intended to include both small molecules and biological molecules. Direct or indirect modulation includes activation or inhibition of EGFR activity or the EGFR signal transduction pathway. In one aspect, inhibition refers to inhibition of the binding of EGFR to an EGFR ligand such as, for example, EGF. In another aspect, inhibition refers to inhibition of the kinase activity of EGFR.

EGFR modulators include, for example, EGFR-specific ligands, small molecule EGFR inhibitors, and EGFR monoclonal antibodies. In one aspect, the EGFR modulator inhibits EGFR activity and/or inhibits the EGFR signal transduction pathway. In another aspect, the EGFR modulator is an EGFR monoclonal antibody that inhibits EGFR activity and/or inhibits the EGFR signal transduction pathway.

EGFR modulators include biological molecules or small molecules. Biological molecules include all lipids and polymers of monosaccharides, amino acids, and nucleotides having a molecular weight greater than 450. Thus, biological molecules include, for example, oligosaccharides and polysaccharides; oligopeptides, polypeptides, peptides, and proteins; and oligonucleotides and polynucleotides. Oligonucleotides and polynucleotides include, for example, DNA and RNA.

Biological molecules further include derivatives of any of the molecules described above. For example, derivatives of biological molecules include lipid and glycosylation derivatives of oligopeptides, polypeptides, peptides, and proteins.

Derivatives of biological molecules further include lipid derivatives of oligosaccharides and polysaccharides, e.g., lipopolysaccharides. Most typically, biological molecules are antibodies, or functional equivalents of antibodies. Functional equivalents of antibodies have binding characteristics comparable to those of antibodies, and inhibit the growth of cells that express EGFR. Such functional equivalents include, for example, chimerized, humanized, and single chain antibodies as well as fragments thereof.

Functional equivalents of antibodies also include polypeptides with amino acid sequences substantially the same as the amino acid sequence of the variable or

hypervariable regions of the antibodies. An amino acid sequence that is substantially the same as another sequence, but that differs from the other sequence by means of one or more substitutions, additions, and/or deletions, is considered to be an equivalent sequence. Preferably, less than 50%, more preferably less than 25%, and still more preferably less than 10%, of the number of amino acid residues in a sequence are substituted for, added to, or deleted from the protein.

The functional equivalent of an antibody is preferably a chimerized or humanized antibody. A chimerized antibody comprises the variable region of a non-human antibody and the constant region of a human antibody. A humanized antibody comprises the hypervariable region (CDRs) of a non-human antibody. The variable region other than the hypervariable region, e.g., the framework variable region, and the constant region of a humanized antibody are those of a human antibody.

Suitable variable and hypervariable regions of non-human antibodies may be derived from antibodies produced by any non-human mammal in which monoclonal antibodies are made. Suitable examples of mammals other than humans include, for example, rabbits, rats, mice, horses, goats, or primates.

Functional equivalents further include fragments of antibodies that have binding characteristics that are the same as, or are comparable to, those of the whole antibody. Suitable fragments of the antibody include any fragment that comprises a sufficient portion of the hypervariable (i.e., complementarity determining) region to bind specifically, and with sufficient affinity, to EGFR tyrosine kinase to inhibit growth of cells that express such receptors.

Such fragments may, for example, contain one or both Fab fragments or the $F(ab')_2$ fragment. Preferably, the antibody fragments contain all six complementarity determining regions of the whole antibody, although functional fragments containing fewer than all of such regions, such as three, four, or five CDRs, are also included.

In one aspect, the fragments are single chain antibodies, or Fv fragments. Single chain antibodies are polypeptides that comprise at least the variable region of the heavy chain of the antibody linked to the variable region of the light chain, with or without an interconnecting linker. Thus, Fv fragment comprises the entire antibody combining site. These chains may be produced in bacteria or in eukaryotic cells.

The antibodies and functional equivalents may be members of any class of immunoglobulins, such as IgG, IgM, IgA, IgD, or IgE, and the subclasses thereof. In one aspect, the antibodies are members of the IgG1 subclass. The functional equivalents may also be equivalents of combinations of any of the above classes and subclasses.

In one aspect, EGFR antibodies can be selected from chimerized, humanized, fully human, and single chain antibodies derived from the murine antibody 225 described in U.S. Patent No. 4,943,533 to Mendelsohn et al.

In another aspect, the EGFR antibody can be selected from the antibodies described in U.S. Patent No. 6,235,883 to Jakobovits et al., U.S. Patent No. 5,558,864 to Bendi et al., and U.S. Patent No. 5,891,996 to Mateo de Acosta del Rio et al.

In addition to the biological molecules discussed above, the EGFR modulators useful in the invention may also be small molecules. Any molecule that is not a biological molecule is considered herein to be a small molecule. Some examples of small molecules include organic compounds, organometallic compounds, salts of organic and organometallic compounds, saccharides, amino acids, and nucleotides. Small molecules further include molecules that would otherwise be considered biological molecules, except their molecular weight is not greater than 450. Thus, small molecules may be lipids, oligosaccharides, oligopeptides, and oligonucleotides and their derivatives, having a molecular weight of 450 or less.

It is emphasized that small molecules can have any molecular weight. They are merely called small molecules because they typically have molecular weights less than 450. Small molecules include compounds that are found in nature as well as synthetic compounds. In one embodiment, the EGFR modulator is a small molecule that inhibits the growth of tumor cells that express EGFR. In another embodiment, the EGFR modulator is a small molecule that inhibits the growth of refractory tumor cells that express EGFR.

Numerous small molecules have been described as being useful to inhibit EGFR. For example, U.S. Patent No. 5,656,655 to Spada et al. discloses styryl substituted heteroaryl compounds that inhibit EGFR. The heteroaryl group is a monocyclic ring with one or two heteroatoms, or a bicyclic ring with 1 to about 4 heteroatoms, the compound being optionally substituted or polysubstituted.

U.S. Patent No. 5,646,153 to Spada et al. discloses bis mono and/or bicyclic aryl heteroaryl, carbocyclic, and heterocarbocyclic compounds that inhibit EGFR.

U.S. Patent No. 5,679,683 to Bridges et al. discloses tricyclic pyrimidine compounds that inhibit the EGFR. The compounds are fused heterocyclic pyrimidine derivatives described at column 3, line 35 to column 5, line 6.

U.S. Patent No. 5,616,582 to Barker discloses quinazoline derivatives that have receptor tyrosine kinase inhibitory activity.

Fry et al., Science 265, 1093-1095 (1994) in Figure 1 discloses a compound having a structure that inhibits EGFR.

Osherov et al. disclose tyrphostins that inhibit EGFR/HER1 and HER 2, particularly those in Tables I, II, III, and IV.

U.S. Patent No. 5,196,446 to Levitzki et al. discloses heteroarylethenediyl or heteroarylethendeiylaryl compounds that inhibit EGFR, particularly from column 2, line 42 to column 3, line 40.

Panek et al., Journal of Pharmacology and Experimental Therapeutics 283, 1433-1444 (1997) discloses a compound identified as PD166285 that inhibits the EGFR, PDGFR, and FGFR families of receptors. PD166285 is identified as 6-(2,6-dichlorophenyl)-2-(4-(2-diethylaminoethyoxy)phenylamino)-8-methyl-8H-pyrido(2,3-d)pyrimidin-7-one having the structure shown in Figure 1 on page 1436.

BIOMARKERS AND BIOMARKER SETS

The invention includes individual biomarkers and biomarker sets having both diagnostic and prognostic value in disease areas in which signaling through EGFR or the EGFR pathway is of importance, e.g., in cancers or tumors, in immunological disorders, conditions or dysfunctions, or in disease states in which cell signaling and/or cellular proliferation controls are abnormal or aberrant. The biomarker sets comprise a plurality of biomarkers such as, for example, a plurality of the biomarkers provided in Table 1, that highly correlate with resistance or sensitivity to one or more EGFR modulators.

The biomarker sets of the invention enable one to predict or reasonably foretell the likely effect of one or more EGFR modulators in different biological systems or for cellular responses. The biomarker sets can be used in *in vitro* assays of

EGFR modulator response by test cells to predict *in vivo* outcome. In accordance with the invention, the various biomarker sets described herein, or the combination of these biomarker sets with other biomarkers or markers, can be used, for example, to predict how patients with cancer might respond to therapeutic intervention with one or more EGFR modulators.

A biomarker set of cellular gene expression patterns correlating with sensitivity or resistance of cells following exposure of the cells to one or more EGFR modulators provides a useful tool for screening one or more tumor samples before treatment with the EGFR modulator. The screening allows a prediction of cells of a tumor sample exposed to one or more EGFR modulators, based on the expression results of the biomarker set, as to whether or not the tumor, and hence a patient harboring the tumor, will or will not respond to treatment with the EGFR modulator.

The biomarker or biomarker set can also be used as described herein for monitoring the progress of disease treatment or therapy in those patients undergoing treatment for a disease involving an EGFR modulator.

The biomarkers also serve as targets for the development of therapies for disease treatment. Such targets may be particularly applicable to treatment of lung disease, such as non-small cell lung cancers or tumors. Indeed, because these biomarkers are differentially expressed in sensitive and resistant cells, their expression patterns are correlated with relative intrinsic sensitivity of cells to treatment with EGFR modulators. Accordingly, the biomarkers highly expressed in resistant cells may serve as targets for the development of new therapies for the tumors which are resistant to EGFR modulators, particularly EGFR inhibitors.

The level of biomarker protein and/or mRNA can be determined using methods well known to those skilled in the art. For example, quantification of protein can be carried out using methods such as ELISA, 2-dimensional SDS PAGE, Western blot, immunopreciptation, immunohistochemistry, fluorescence activated cell sorting (FACS), or flow cytometry. Quantification of mRNA can be carried out using methods such as PCR, array hybridization, Northern blot, in-situ hybridization, dot-blot, Taqman, or RNAse protection assay.

MICROARRAYS

The invention also includes specialized microarrays, e.g., oligonucleotide microarrays or cDNA microarrays, comprising one or more biomarkers, showing expression profiles that correlate with either sensitivity or resistance to one or more EGFR modulators. Such microarrays can be employed in *in vitro* assays for assessing the expression level of the biomarkers in the test cells from tumor biopsies, and determining whether these test cells are likely to be resistant or sensitive to EGFR modulators. For example, a specialized microarray can be prepared using all the biomarkers, or subsets thereof, as described herein and shown in Table 1. Cells from a tissue or organ biopsy can be isolated and exposed to one or more of the EGFR modulators. Following application of nucleic acids isolated from both untreated and treated cells to one or more of the specialized microarrays, the pattern of gene expression of the tested cells can be determined and compared with that of the biomarker pattern from the control panel of cells used to create the biomarker set on the microarray. Based upon the gene expression pattern results from the cells that underwent testing, it can be determined if the cells show a resistant or a sensitive profile of gene expression. Whether or not the tested cells from a tissue or organ biopsy will respond to one or more of the EGFR modulators and the course of treatment or therapy can then be determined or evaluated based on the information gleaned from the results of the specialized microarray analysis.

ANTIBODIES

The invention also includes antibodies, including polyclonal or monoclonal, directed against one or more of the polypeptide biomarkers. Such antibodies can be used in a variety of ways, for example, to purify, detect, and target the biomarkers of the invention, including both *in vitro* and *in vivo* diagnostic, detection, screening, and/or therapeutic methods.

KITS

The invention also includes kits for determining or predicting whether a patient would be susceptible or resistant to a treatment that comprises one or more EGFR modulators. The patient may have a cancer or tumor such as, for example, a

non-small cell lung cancer or tumor. Such kits would be useful in a clinical setting for use in testing a patient's biopsied tumor or other cancer samples, for example, to determine or predict if the patient's tumor or cancer will be resistant or sensitive to a given treatment or therapy with an EGFR modulator. The kit comprises a suitable container that comprises: one or more microarrays, e.g., oligonucleotide microarrays or cDNA microarrays, that comprise those biomarkers that correlate with resistance and sensitivity to EGFR modulators, particularly EGFR inhibitors; one or more EGFR modulators for use in testing cells from patient tissue specimens or patient samples; and instructions for use. In addition, kits contemplated by the invention can further include, for example, reagents or materials for monitoring the expression of biomarkers of the invention at the level of mRNA or protein, using other techniques and systems practiced in the art such as, for example, RT-PCR assays, which employ primers designed on the basis of one or more of the biomarkers described herein, immunoassays, such as enzyme linked immunosorbent assays (ELISAs), immunoblotting, e.g., Western blots, or *in situ* hybridization, and the like, as further described herein.

APPLICATION OF BIOMARKERS AND BIOMARKER SETS

The biomarkers and biomarker sets may be used in different applications. Biomarker sets can be built from any combination of biomarkers listed in Table 1 to make predictions about the likely effect of any EGFR modulator in different biological systems. The various biomarkers and biomarkers sets described herein can be used, for example, as diagnostic or prognostic indicators in disease management, to predict how patients with cancer might respond to therapeutic intervention with compounds that modulate the EGFR, and to predict how patients might respond to therapeutic intervention that modulates signaling through the entire EGFR regulatory pathway.

The biomarkers have both diagnostic and prognostic value in diseases areas in which signaling through EGFR or the EGFR pathway is of importance, e.g., in immunology, or in cancers or tumors in which cell signaling and/or proliferation controls have gone awry.

In accordance with the invention, cells from a patient tissue sample, e.g., a tumor or cancer biopsy, can be assayed to determine the expression pattern of one or more biomarkers prior to treatment with one or more EGFR modulators. In one aspect, the tumor or cancer is NSCLC. Success or failure of a treatment can be determined based on the biomarker expression pattern of the cells from the test tissue (test cells), e.g., tumor or cancer biopsy, as being relatively similar or different from the expression pattern of a control set of the one or more biomarkers. Thus, if the test cells show a biomarker expression profile which corresponds to that of the biomarkers in the control panel of cells which are sensitive to the EGFR modulator, it is highly likely or predicted that the individual's cancer or tumor will respond favorably to treatment with the EGFR modulator. By contrast, if the test cells show a biomarker expression pattern corresponding to that of the biomarkers of the control panel of cells which are resistant to the EGFR modulator, it is highly likely or predicted that the individual's cancer or tumor will not respond to treatment with the EGFR modulator.

The invention also provides a method of monitoring the treatment of a patient having a disease treatable by one or more EGFR modulators. The isolated test cells from the patient's tissue sample, e.g., a tumor biopsy or tumor sample, can be assayed to determine the expression pattern of one or more biomarkers before and after exposure to an EGFR modulator wherein, preferably, the EGFR modulator is an EGFR inhibitor. The resulting biomarker expression profile of the test cells before and after treatment is compared with that of one or more biomarkers as described and shown herein to be highly expressed in the control panel of cells that are either resistant or sensitive to an EGFR modulator. Thus, if a patient's response is sensitive to treatment by an EGFR modulator, based on correlation of the expression profile of the one or biomarkers, the patient's treatment prognosis can be qualified as favorable and treatment can continue. Also, if, after treatment with an EGFR modulator, the test cells don't show a change in the biomarker expression profile corresponding to the control panel of cells that are sensitive to the EGFR modulator, it can serve as an indicator that the current treatment should be modified, changed, or even discontinued. This monitoring process can indicate success or failure of a patient's treatment with an EGFR modulator and such monitoring processes can be repeated as necessary or desired.

The biomarkers of the invention can be used to predict an outcome prior to having any knowledge about a biological system. Essentially, a biomarker can be considered to be a statistical tool. Biomarkers are useful primarily in predicting the phenotype that is used to classify the biological system.

Although the complete function of all of the biomarkers are not currently known, some of the biomarkers are likely to be directly or indirectly involved in the EGFR signaling pathway. In addition, some of the biomarkers may function in metabolic or other resistance pathways specific to the EGFR modulators tested. Notwithstanding, knowledge about the function of the biomarkers is not a requisite for determining the accuracy of a biomarker according to the practice of the invention.

EXAMPLES:

EXAMPLE 1 - Identification of Biomarkers

The biomarkers of Table 1 were identified using three particular approaches. The transcriptional profiling data from primary tumors and cell lines was examined to identify genes with expression that is highly variable across the tumors and cell lines. In addition, attempts were made to determine the IC_{50} on a panel of cell lines in order to identify genes whose expression profiles correlate with sensitive/resistant classification based on IC_{50} values. Furthermore, cell lines and xenograft models were treated with the chimeric EGFR antibody cetuximab (marketed as Erbitux®) and the small molecule EGFR inhibitor gefitinib to identify genes that are modulated by EGFR inhibitors.

NSCLC tumors and patients:

RNAs from twenty-nine NSCLC adenocarcinoma tumors were obtained (Ardais Corporation, Somerville, MA). Adenocarcinomas are the most common sub-type of NSCLC. The median age of the patients was 65 years (range: 43-80 years). The tumors belonged to all size ranges T1 – T4 and all stages ranging from Stage IA to Stage IV according to the AJCC classification.

Determination of Relative Drug Sensitivity in NSCLC Cell Lines:

The NSCLC cell lines were grown using standard cell culture conditions:

DMEM supplemented to contain 10% fetal bovine serum, 100 IU/ml penicillin, 100 mg/ml streptomycin and 2 mM L-glutamine (all from Invitrogen Life Technologies, Carlsbad, CA). Fourteen non-small cell lung cancer cell lines were examined for their sensitivity to EGFR inhibitor monoclonal antibody cetuximab. Cytotoxicity was assessed in cells by BrdU Cell Proliferation colorimetric ELISA (Roche Applied Science, Indianapolis, IN). This is a colorimetric immunoassay for the quantification of cell proliferation based on the measurement of BrdU incorporation during DNA synthesis. To carry out the assays, the NSCLC cells were plated at 2500-5000 cells/well in 96 well microtiter plates and 24 hours later diluted monoclonal antibody drug was added. The concentrations for the EGFR inhibitor cetuximab used in the cytotoxicity assays was 5 μg/ml, 4 μg/ml, 2 μg/ml, 1 μg/ml and 0.5 μg/ml. The cells were incubated at 37 °C for 48 hours at which time the BrdU labeling reagent was added. After two hours the labeling medium was removed and cells were fixed and the DNA was denatured using a FixDenat solution. The anti-BrdU antibody conjugated with peroxidase was added and immune complexes were detected by the subsequent substrate reaction. The reaction product was quantified by measuring the absorbance of the samples in an ELISA reader at 450 nm. The greater the absorbency, the greater the number of live cells. Only two of the fourteen cell lines tested had an IC_{50} between 4 and 5μg/ml. The IC_{50} is the drug concentration required to inhibit cell proliferation to 50% of that of untreated cells. Three to six independent BrdU assays were performed for each cell line.

Resistance/sensitivity classification:

FIG. 1 shows the mRNA level of the epidermal growth factor receptor gene as determined by expression profiling of fourteen NSCLC cell lines that were tested in the BrdU assays described above. Cell lines are shown in order of increasing sensitivity to cetuximab. As shown in FIG. 1, there is no correlation between EGFR level and sensitivity to cetuximab. Of the fourteen NSCLC cell lines tested, ChagoK1 and L2987 were the only two cell lines that consistently showed ≥ 50% inhibition of cell proliferation at the IC_{50} concentration of cetuximab. Cell lines SW900, Calu6, SK-MES1, H838 and H661 showed significantly lower than 50% inhibition of cell proliferation at the doses of cetuximab that were tested. The remaining cell lines

LX1, H522, H441, H226, A549, SK-LU1 and H2347 showed no inhibition of cell proliferation at the doses of cetuximab that were tested. For the analysis, cell lines ChagoK1 and L2987 were defined as sensitive and the remaining twelve cell lines were defined as resistant.

Gene Expression Profiling:

RNA for the NSCLC adenocarcinomas was purchased from a commercial vendor as described above. For the NSCLC cell lines, RNA was isolated from 50-70% confluent cells using the RNeasy kits (Qiagen, Valencia, CA). The quality of RNA was checked by measuring the 28S:18: ribosomal RNA ratio using an Agilent 2100 Bioanalyzer (Agilent Technologies, Rockville, MD). Concentration of total RNA was determined spectrophotometrically. 5 or 10 ug of total RNA was used to prepare biotinylated probes according to the Affymetrix Genechip Expression Analysis Technical Manual. Targets were hybridized to human HG-U133A gene chips according to the manufacturer's instructions. Data were preprocessed using the MAS 5.0 software (Affymetrix, Santa Clara, CA). The trimmed mean intensity for each chip was scaled to 1,500 to account for minor differences in global chip intensity so that the overall expression level for each sample is comparable.

Data Analysis

All 22,215 probes (gene sequences) present on the U133A chip were considered as potential predictive biomarkers. To restrict the analysis to gene sequences expressed in at least two of the twenty nine NSCLC tumors, gene sequences with Affymetrix MAS5.0 $p > 0.04$ in at least two tumors or cell lines were removed leaving 14,354 and 13,909 gene sequences, respectively (FIG. 2).

Next, to identify genes with variable expression in lung tumors (and therefore more likely to be able to correlate with variability in response to treatment), a variance metric (the Weighted spread (90-10) metric) (WSpread (90-10) metric) was used to calculate the variance of probe sets in the tumor and cell line expression profiling data.

$$\text{Weighted spread} = \frac{I\,90\text{th percentile} - I\,10\text{th percentile}}{\sqrt{I\text{median}}}$$

I = Signal intensity from expression profiling data

Gene sequences with a WSpread (90-10) metric < 30 were removed leaving 4167 gene sequences in the adenocarcinoma tumors (FIG. 3) and 4274 gene sequences in the cell lines (FIG. 4).

Next, the same expression filter was applied to the remaining 4167 gene sequences using the NSCLC cell line data, resulting in 3572 gene sequences for analysis. This was followed by the application of the same variance metric filter leaving 2496 gene sequences for analysis. Of the 2496 gene sequences, 776 genes sequences ranked in the top 1000 in the cell line variance analysis. These 776 sequences were chosen for further statistical analysis. The 776 gene sequences were subjected to a two-sided unequal variance t-test using the resistance/sensitivity classifications of the cell lines described above (FIG. 1). 147 gene sequences showed a significantly different expression profile between the sensitive and resistant cell lines with a p-value of <0.05 (FIG. 5). Table 1 provides a list of the 147 gene sequences identified using the two-sided unequal variance T-test. These 147 gene sequences (probe sets) represent 124 biomarkers with regard to the Unigene Titles.

A variation of the gene filtering scheme illustrated in FIG. 1 was conducted and is illustrated in FIG. 2. In this scheme, 343 gene sequences ranked in the top 1000 in both the tumor and cell line variance analysis, a total of 343 out of the 776 genes sequences, were subjected to a two-sided unequal variance T-test. 59 gene sequences showed a significantly different expression profile between the sensitive and resistant cell lines with a p-value of <0.05. These 59 biomarkers are provided in Table 1 as the first 59 biomarkers, i.e., SEQ ID NOS:1-59 and 148-206.

EXAMPLE 2 – Experimental Validation of Biomarker Candidates: Cell line induction studies

Regulation by EGFR inhibitors in drug treated cell lines would lend additional support to the candidate biomarkers as being predictive of response. Induction

experiments were carried out in two sensitive cell lines ChagoK1 (sensitive to cetuximab and gefitinib) and L2987 (sensitive to cetuximab, resistant to gefitinib). Induction experiments were also carried out in four cell lines that were resistant to both EGFR inhibitors: A549 and H226 (EGFR+) and LX-1 and H522 (EGFR negative) cell lines.

Cells were seeded in 6-well tissue culture dishes in DMEM supplemented with 10% FBS (Invitrogen, Carlsbad, CA). Twenty-four hours later the cells were switched to DMEM containing 0.5% FBS. The next day cells were treated with either 4μg/ml cetuximab or 1μM gefitinib. Twenty-four hours later cells were stimulated with 100ng/ml human recombinant epidermal growth factor EGF (Biosource International, Camarillo, CA) for 6 hours. The cells were lysed directly in the culture dish and RNA isolation was carried out using the RNeasy mini kit (Qiagen, Valencia, CA). Profiling was done on U133A GeneChips (Affymetrix, Santa Clara, CA). Data was analyzed using GeneChip® Expression Analysis software MAS 5.0 (Affymetrix, Santa Clara, California). Anova analysis of profiling data was done with PartekPro pattern recognition software (Partek, St. Charles, MS) using quantile normalized Affymetrix MAS5.0 values for signal intensity.

Of the 147 probe sets examined, 21 probe sets representing 18 different biomarkers (provided below in Table 3) were highly regulated (Bonferroni $p<0.05$ in Anova analysis) upon EGFR inhibitor treatment and/or EGF stimulation in the sensitive cell lines.

TABLE 3 - Biomarkers Highly Regulated by EGFR Inhibitor Treatment and/or EGF Stimulation in the Sensitive Cell Lines

Unigene title and SEQID NO:	Affymetrix Description	Affymetrix Probe Set
DKK1: dickkopf homolog 1 (LOC22943) SEQ ID NOS: 7 (DNA) and 154 (amino acid)	gb:NM_012242.1 /DEF=Homo sapiens dickkopf (Xenopus laevis) homolog 1 (DKK1), mRNA. /FEA=mRNA /GEN=DKK1 /PROD=dickkopf (Xenopus laevis) homolog 1 /DB_XREF=gi:7110718 /UG=Hs.40499 dickkopf (Xenopus laevis) homolog 1 /FL=gb:AF127563.1 gb:AF177394.1 gb:NM_012242.1	204602_at

S100A9: S100 calcium-binding protein A9 (LOC6280) SEQ ID NOS: 10 (DNA) and 157 (amino acid)	gb:NM_002965.2 /DEF=Homo sapiens S100 calcium-binding protein A9 (calgranulin B) (S100A9), mRNA. /FEA=mRNA /GEN=S100A9 /PROD=S100 calcium-binding protein A9 /DB_XREF=gi:9845520 /UG=Hs.112405 S100 calcium-binding protein A9 (calgranulin B) /FL=gb:M26311.1 gb:NM_002965.2	203535_at
SFN: stratifin (LOC2810) SEQ ID NOS: 11 (DNA) and 158 (amino acid)	Cluster Incl. X57348:H.sapiens mRNA (clone 9112) /cds=(165,911) /gb=X57348 /gi=23939 /ug=Hs.184510 /len=1407	33322_i_at
PBEF: pre-B-cell colony-enhancing factor isoform a (LOC10135) SEQ ID NOS: 36 (DNA) and 183 (amino acid)	Consensus includes gb:BF575514 /FEA=EST /DB_XREF=gi:11649318 /DB_XREF=est:602133090F1 /CLONE=IMAGE:4288079 /UG=Hs.239138 pre-B-cell colony-enhancing factor /FL=gb:U02020.1 gb:NM_005746.1	217738_at
SERPINE2: plasminogen activator inhibitor type 1, member 2 (LOC5270) SEQ ID NOS: 38 (DNA) and 185 (amino acid)	Consensus includes gb:AL541302 /FEA=EST /DB_XREF=gi:12872241 /DB_XREF=est:AL541302 /CLONE=CS0DE006YI10 (5 prime) /UG=Hs.21858 trinucleotide repeat containing 3	212190_at
SFN: stratifin (LOC2810) SEQ ID NOS: 41 (DNA) and 188 (amino acid)	Cluster Incl. X57348:H.sapiens mRNA (clone 9112) /cds=(165,911) /gb=X57348 /gi=23939 /ug=Hs.184510 /len=1407	33323_r_at
IL8: interleukin 8 (LOC3576) SEQ ID NOS: 44 (DNA) and 191 (amino acid)	gb:AF043337.1 /DEF=Homo sapiens interleukin 8 C-terminal variant (IL8) mRNA, complete cds. /FEA=mRNA /GEN=IL8 /PROD=interleukin 8 C-terminal variant /DB_XREF=gi:12641914 /UG=Hs.624 interleukin 8 /FL=gb:AF043337.1	211506_s_at

CTSC: cathepsin C isoform a preproprotein (LOC1075) SEQ ID NOS: 46 (DNA) and 193 (amino acid)	gb:NM_001814.1 /DEF=Homo sapiens cathepsin C (CTSC), mRNA. /FEA=mRNA /GEN=CTSC /PROD=cathepsin C /DB_XREF=gi:4503140 /UG=Hs.10029 cathepsin C /FL=gb:NM_001814.1	201487_at
TXNIP: thioredoxin interacting protein (LOC10628) SEQ ID NOS: 50 (DNA) and 197 (amino acid)	Consensus includes gb:AA812232 /FEA=EST /DB_XREF=gi:2881843 /DB_XREF=est:ob84h09.s1 /CLONE=IMAGE:1338113 /UG=Hs.179526 upregulated by 1,25-dihydroxyvitamin D-3 /FL=gb:NM_006472.1 gb:S73591.1	201008_s_at
SAT: spermidine/spermine N1-acetyltransferase (LOC6303) SEQ ID NOS: 54 (DNA) and 201 (amino acid)	gb:M55580.1 /DEF=Human spermidinespermine N1-acetyltransferase mRNA, complete cds. /FEA=mRNA /GEN=spermidinespermine N1-acetyltransferase /PROD=spermidinespermine N1-acetyltransferase /DB_XREF=gi:338335 /UG=Hs.28491 spermidinespermine N1-acetyltransferase /FL=gb:M55580.1	210592_s_at
TXNIP: thioredoxin interacting protein (LOC10628) SEQ ID NOS: 57 (DNA) and 204 (amino acid)	gb:NM_006472.1 /DEF=Homo sapiens upregulated by 1,25-dihydroxyvitamin D-3 (VDUP1), mRNA. /FEA=mRNA /GEN=VDUP1 /PROD=upregulated by 1,25-dihydroxyvitamin D-3 /DB_XREF=gi:5454161 /UG=Hs.179526 upregulated by 1,25-dihydroxyvitamin D-3 /FL=gb:NM_006472.1 gb:S73591.1	201010_s_at
TENS1: tensin-like SH2 domain-containing 1 (LOC64759) SEQ ID NOS: 66 (DNA) and 213 (amino acid)	gb:NM_022748.1 /DEF=Homo sapiens hypothetical protein FLJ13732 similar to tensin (FLJ13732), mRNA. /FEA=mRNA /GEN=FLJ13732 /PROD=hypothetical protein FLJ13732 similar to tensin /DB_XREF=gi:12232408 /UG=Hs.12210 hypothetical protein FLJ13732 similar to tensin /FL=gb:NM_022748.1	217853_at
STK17A: serine/threonine kinase 17a (apoptosis-inducing) (LOC9263) SEQ ID NOS: 69 (DNA) and 216 (amino acid)	Consensus includes gb:AW194730 /FEA=EST /DB_XREF=gi:6473630 /DB_XREF=est:xn43d11.x1 /CLONE=IMAGE:2696469 /UG=Hs.9075 serinethreonine kinase 17a (apoptosis-inducing) /FL=gb:AB011420.1 gb:NM_004760.1	202693_s_at

TUBB-5: tubulin beta-5 (LOC84617) SEQ ID NOS: 84 (DNA) and 231 (amino acid)	gb:BC002654.1 /DEF=Homo sapiens, Similar to tubulin, beta, 4, clone MGC:4083, mRNA, complete cds. /FEA=mRNA /PROD=Similar to tubulin, beta, 4 /DB_XREF=gi:12803638 /UG=Hs.274398 Homo sapiens, Similar to tubulin, beta, 4, clone MGC:4083, mRNA, complete cds /FL=gb:BC002654.1	209191_at
TYMS: thymidylate synthetase (LOC7298) SEQ ID NOS: 85 (DNA) and 232 (amino acid)	gb:NM_001071.1 /DEF=Homo sapiens thymidylate synthetase (TYMS), mRNA. /FEA=mRNA /GEN=TYMS /PROD=thymidylate synthetase /DB_XREF=gi:4507750 /UG=Hs.82962 thymidylate synthetase /FL=gb:BC002567.1 gb:NM_001071.1	202589_at
RAI14: retinoic acid induced 14 (LOC26064) SEQ ID NOS: 97 (DNA) and 244 (amino acid)	gb:NM_015577.1 /DEF=Homo sapiens novel retinal pigment epithelial gene (NORPEG), mRNA. /FEA=mRNA /GEN=NORPEG /PROD=DKFZP564G013 protein /DB_XREF=gi:13470085 /UG=Hs.15165 novel retinal pigment epithelial gene /FL=gb:NM_015577.1 gb:AF155135.1	202052_s_at
CALD1: caldesmon 1 isoform 3 (LOC800) SEQ ID NOS: 106 (DNA) and 253 (amino acid)	Consensus includes gb:AL583520 /FEA=EST /DB_XREF=gi:12952562 /DB_XREF=est:AL583520 /CLONE=CS0DC024YE13 (5 prime) /UG=Hs.182183 Homo sapiens mRNA for caldesmon, 3 UTR	212077_at
PALM2: paralemmin 2 (LOC114299) SEQ ID NOS: 115 (DNA) and 262 (amino acid)	gb:NM_007203.1 /DEF=Homo sapiens A kinase (PRKA) anchor protein 2 (AKAP2), mRNA. /FEA=mRNA /GEN=AKAP2 /PROD=A kinase (PRKA) anchor protein 2 /DB_XREF=gi:6005708 /UG=Hs.42322 A kinase (PRKA) anchor protein 2 /FL=gb:AB023137.1 gb:NM_007203.1	202760_s_at
TPM1 : tropomyosin 1 (alpha) (LOC7168) SEQ ID NOS: 125 (DNA) and 272 (amino acid)	gb:Z24727.1 /DEF=H.sapiens tropomyosin isoform mRNA, complete CDS. /FEA=mRNA /PROD=tropomyosin isoform /DB_XREF=gi:854188 /UG=Hs.77899 tropomyosin 1 (alpha) /FL=gb:Z24727.1	210986_s_at
TPM1 : tropomyosin 1 (alpha) (LOC7168) SEQ ID NOS: 137 (DNA) and 284 (amino acid)	gb:M19267.1 /DEF=Human tropomyosin mRNA, complete cds. /FEA=mRNA /DB_XREF=gi:339943 /UG=Hs.77899 tropomyosin 1 (alpha) /FL=gb:M19267.1	210987_x_at

TUBB: tubulin, beta polypeptide (LOC7280) SEQ ID NOS: 147 (DNA) and 294 (amino acid)	gb:NM_001069.1 /DEF=Homo sapiens tubulin, beta polypeptide (TUBB), mRNA. /FEA=mRNA /GEN=TUBB /PROD=tubulin, beta polypeptide /DB_XREF=gi:4507728 /UG=Hs.179661 tubulin, beta polypeptide /FL=gb:BC001194.1 gb:NM_001069.1	204141_at

It appears that these biomarkers are likely to be directly or indirectly involved in the EGFR signaling pathway, based on their expression modulation by EGF and / or EGFR inhibitor treatment.

EXAMPLE 3 – Experimental Validation of Biomarker Candidates: Drug treatment studies in lung xenograft models

Regulation by EGFR inhibitors in lung xenograft models would lend additional support to the candidate markers, as being predictive of response. Drug treatment experiments were carried out in the L2987 (sensitive to cetuximab and gefitinib), A549 (borderline sensitive to cetuximab and gefitinib), and LX1 (resistant to cetuximab and gefitinib) lung xenograft models.

In Vivo Antitumor Testing

Tumors were propagated in nude mice as subcutaneous (sc) transplants using tumor fragments obtained from donor mice. Tumor passage occurred approximately every two to four weeks. Tumors were then allowed to grow to the pre-determined size window (usually between 100-200 mg, tumors outside the range were excluded) and animals were evenly distributed to various treatment and control groups. Animals were treated with cetuximab (1 mg/mouse, q3d X 10, 14; ip) or gefitinib (200mg/kg, q1dX14, 14; po). Treated animals were checked daily for treatment related toxicity/mortality. Each group of animals was weighed before the initiation of treatment (Wt1) and then again following the last treatment dose (Wt2). The difference in body weight (Wt2-Wt1) provided a measure of treatment-related toxicity. Tumor response was determined by measurement of tumors with a caliper twice a week, until the tumors reached a predetermined target size of 1 gm or became necrotic. Tumor weights (mg) were estimated from the formula:

$$\text{Tumor weight} = (\text{length} \times \text{width}^2)/2$$

Antitumor activity was determined in terms of primary tumor growth inhibition. This was determined in two ways: (i) calculating the relative median tumor weight (MTW) of treated (T) and control (C) mice at various time points (effects were expressed as %T/C); and (ii) calculating the tumor growth delay (T-C value), defined as the difference in time (days) required for the treated tumors (T) to reach a predetermined target size compared to those of the control group (C). Statistical evaluations of data were performed using Gehan's generalized Wilcoxon test for comparisons of time to reach tumor target size (Gehan 1965). Statistical significance was declared at $p < 0.05$. Antitumor activity was defined as a continuous MTW %T/C $\leq$ 50% for at least 1 tumor volume doubling time (TVDT) any time after the start of treatment, where TVDT (tumor volume doubling time) = median time (days) for control tumors to reach target size – median time (days) for control tumors to reach half the target size. In addition, treatment groups had to be accompanied by a statistically significant tumor growth delay (T-C value) ($p < 0.05$) to be termed active.

Treated animals were checked daily for treatment related toxicity/mortality. When death occurred, the day of death was recorded. Treated mice dying prior to having their tumors reach target size were considered to have died from drug toxicity. No control mice died bearing tumors less than target size. Treatment groups with more than one death caused by drug toxicity were considered to have had excessively toxic treatments and their data were not included in the evaluation of the compound's antitumor efficacy.

Drug treatment experiments

L2987 and A549 xenograft animals were dosed with a single dose of either (1) 1 mg/mouse cetuximab, ip; (2) 250mg/kg gefitinib, po; (3) PEG400/H_2O vehicle, po or 4) PBS vehicle, ip. Each dose was given to three independent mice. At 3h and 24h post-treatment the animals were sacrificed and tumors were excised and immediately placed into RNA*later* solution (Qiagen, Valencia, CA).

RNA was isolated from the tumors using the RNeasy kits (Qiagen, Valencia, CA). The quality and concentration of total RNA was determined as described previously. Profiling was done on U133A GeneChips (Affymetrix, Santa Clara, CA).

Data was analyzed using GeneChip® Expression Analysis software MAS 5.0 (Affymetrix, Santa Clara, California). Anova analysis of profiling data was done with PartekPro pattern recognition software (Partek, St. Charles, MS) using quantile normalized Affymetrix MAS5.0 values for signal intensity.

Out of 147 probesets examined, 4 probesets representing 3 genes are significantly regulated ($p<0.005$ in Anova analysis) upon EGFR inhibitor treatment in the sensitive L2987 xenograft but not in the borderline sensitive A549 xenograft. The three genes are jumping translocation breakpoint (JTB), 3-phosphoadenosine 5-phosphosulfate synthase 2 (PAPSS2) and serine protease inhibitor, Kunitz type 1 (SPINT1). It appears that these biomarkers are likely to be directly or indirectly involved in the EGFR signaling pathway, based on their expression modulation by EGFR inhibitor treatment.

EXAMPLE 4 - Immunohistochemistry (IHC) assays in clinical samples

Of the 147 probe sets identified preclinically, S100A9 (Calgranulin B) was chosen to examine whether there was any correlation between expression of a particular protein in the clinical samples and Best Clinical Response data.

Basic IHC Method

Formalin-fixed, paraffin-embedded tissues were available on slides in 5 μm sections. The sections were deparaffinized with standard xylene and hydrated through graded alcohols into water. Antigen retrieval was performed using proteinase K. Staining was done at room temperature on an automatic staining workstation TechMate 1000 (BioTek Solutions/Ventana Medical Systems, Tucson, AZ) by using the Envision peroxidase mouse system (DakoCytomation, Carpinteria, CA). Slides were placed three times for 2.5 minutes each in a hydrogen peroxide blocking medium and then allowed to react with mouse anti-human Calgranulin B monoclonal antibody (Bachem Biomedical, Germany) for 60 minutes. Immunodetection was performed with the Envision system by placing slides three times for 5 minutes each in diaminobenzidine (DAB) chromogen substrate. Counterstaining with hematoxylin for 1 minute was the final step. After staining, slides were dehydrated through an alcohol series to absolute ethanol followed by xylene rinses. Slides were permanently

coverslipped with glass coverslips and permount medium. Slides were examined under a microscope to assess staining. Positive staining is indicated by the presence of a dark brown chromogen (DAB-Horse Radish Peroxidase reaction product). Hematoxylin counterstain provides a blue nuclear stain to assess cell and tissue morphology. Appropriate positive and negative controls were used. The slides were viewed randomly, without clinical data, by two independent evaluators and scored. A simple scoring system was used to reflect whether a tissue is positive or negative for the marker and to indicate the relative level of staining. A scoring scheme of negative, low, moderate or high was used to indicate the relative percentage of tumor cells staining within the tissues (FIG. 7). The scoring system simply provides an indication of relative expression of a target from tissue to tissue.

Clinical materials and criteria for response

Formalin-fixed paraffin embedded lung tumor slides were obtained from patients enrolled in a phase II trial of cetuximab. In this trial, cetuximab was used as a single agent therapy for recurrent non-small-cell lung cancer patients (unpublished). The best overall response was recorded from the start of the treatment until disease progression or recurrence. Assessment of response was performed using the RECIST criteria (Response Evaluation Criteria in Solid Tumors, Tsuchida and Therasse, 2001). A partial response (PR) described at least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD. Progressive disease (PD) referred to a 20% or greater increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of new lesions. Stable Disease (SD) was used to describe neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

Calgranulin B IHC assay on clinical FFPET slides

Calgranulin B IHC assay was performed on FFPET slides from 39 patients enrolled in the phase II trial of cetuximab in recurrent NSCLC patients (Table 4). Of the 39 patients, 10 were excluded from further analysis because there was no detectable tumor specimen on the slide. The remaining 29 patients that were scored for Calgranulin B staining comprised of 2 PR, 12 SD and 15 PD non-responders

based on the clinical response data. The 39 samples used in this IHC analysis were derived from patients for whom tissue samples were available and from whom an informed consent could be obtained. It should be noted that the response data shown here may not reflect the response rate in the entire study.

Of the 29 patients' slides, 22 were scored as 0, 3 were scored as 0.5+, 3 were scored as 1+ and 1 slide was scored as 2+. Overall 24 % of the patients tested were positive for Calgranulin B staining (Table 4).

TABLE 4 - IHC Assay Results

PROGRESSIVE DISEASE			DISEASE STABILIZATION		
ID	Best Clinical Response	IHC	ID	Best Clinical Response	IHC
L8	PD	negative	L10	SD	negative
L11	PD	negative	L13	SD	positive
L12	PD	positive	L40	SD	negative
L14	PD	negative	L24	SD	negative
L15	PD	negative	L27	SD	positive
L18	PD	negative	L47	SD	positive
L20	PD	negative	L28	SD	negative
L41	PD	negative	L3	SD	negative
L42	PD	negative	L4	SD	negative
L44	PD	negative	L6	SD	positive
L16	PD	negative	L34	SD	negative
L5	PD	negative	L39	SD	positive
L33	PD	negative	L1	PR	positive
L37	PD	negative	L2	PR	negative
L23B	PD	negative			

The results are summarized in Table 5 below.

TABLE 5 - IHC Assay Results Summary

	# responders (PR +SD)	# non-responders
Calgranulin B +	6	1
Calgranulin B -	9	13

Of the 7 patients that were Calgranulin B positive, 6 had disease stabilization and 1 was a non-responder having progressive disease (Table 5). The sensitivity of the

assay to identify potential responders is 40% [6/ (6+9)] and the specificity is 93% [13/ (13+1)].

The positive predictive value of a Calgranulin B IHC assay to identify potential responders is 86% [6/ (6+1)] and the negative predictive value = 59% [13/(13+9)], {Chi square p value =0.03}.

Although the data set is small, these results indicate a trend for Calgranulin B positive patients to have disease stabilization.

EXAMPLE 5 - PRODUCTION OF ANTIBODIES AGAINST THE BIOMARKERS

Antibodies against the biomarkers can be prepared by a variety of methods. For example, cells expressing a biomarker polypeptide can be administered to an animal to induce the production of sera containing polyclonal antibodies directed to the expressed polypeptides. In one aspect, the biomarker protein is prepared and isolated or otherwise purified to render it substantially free of natural contaminants, using techniques commonly practiced in the art. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity for the expressed and isolated polypeptide.

In one aspect, the antibodies of the invention are monoclonal antibodies (or protein binding fragments thereof). Cells expressing the biomarker polypeptide can be cultured in any suitable tissue culture medium, however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented to contain 10% fetal bovine serum (inactivated at about 56 °C), and supplemented to contain about 10 g/l nonessential amino acids, about 1,00 U/ml penicillin, and about 100 µg/ml streptomycin.

The splenocytes of immunized (and boosted) mice can be extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line can be employed in accordance with the invention, however, it is preferable to employ the parent myeloma cell line (SP2/0), available from the ATCC (Manassas, VA). After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (1981, *Gastroenterology*, 80:225-232). The hybridoma cells obtained through such a selection are then assayed

to identify those cell clones that secrete antibodies capable of binding to the polypeptide immunogen, or a portion thereof.

Alternatively, additional antibodies capable of binding to the biomarker polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens and, therefore, it is possible to obtain an antibody that binds to a second antibody. In accordance with this method, protein specific antibodies can be used to immunize an animal, preferably a mouse. The splenocytes of such an immunized animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones that produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce the formation of further protein-specific antibodies.

EXAMPLE 6 - IMMUNOFLUORESCENCE ASSAYS

The following immunofluorescence protocol may be used, for example, to verify EGFR biomarker protein expression on cells or, for example, to check for the presence of one or more antibodies that bind EGFR biomarkers expressed on the surface of cells. Briefly, Lab-Tek II chamber slides are coated overnight at 4 °C with 10 micrograms/milliliter (μg/ml) of bovine collagen Type II in DPBS containing calcium and magnesium (DPBS++). The slides are then washed twice with cold DPBS++ and seeded with 8000 CHO-CCR5 or CHO pC4 transfected cells in a total volume of 125 μl and incubated at 37 °C in the presence of 95% oxygen / 5% carbon dioxide.

The culture medium is gently removed by aspiration and the adherent cells are washed twice with DPBS++ at ambient temperature. The slides are blocked with DPBS++ containing 0.2% BSA (blocker) at 0-4 °C for one hour. The blocking solution is gently removed by aspiration, and 125 μl of antibody containing solution (an antibody containing solution may be, for example, a hybridoma culture supernatant which is usually used undiluted, or serum/plasma which is usually diluted, e.g., a dilution of about 1/100 dilution). The slides are incubated for 1 hour at 0-4 °C. Antibody solutions are then gently removed by aspiration and the cells are

washed five times with 400 µl of ice cold blocking solution. Next, 125 µl of 1 µg/ml rhodamine labeled secondary antibody (e.g., anti-human IgG) in blocker solution is added to the cells. Again, cells are incubated for 1 hour at 0-4 °C.

The secondary antibody solution is then gently removed by aspiration and the cells are washed three times with 400 µl of ice cold blocking solution, and five times with cold DPBS++. The cells are then fixed with 125 µl of 3.7% formaldehyde in DPBS++ for 15 minutes at ambient temperature. Thereafter, the cells are washed five times with 400 µl of DPBS++ at ambient temperature. Finally, the cells are mounted in 50% aqueous glycerol and viewed in a fluorescence microscope using rhodamine filters.